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# CONTENTS

No. 1. MARCH 1942

The Effect of Hypoglycemia on the Electroencephalogram at Varying Degrees of Oxygenation of the Blood. <i>E. Gellhorn and M. Kessler</i> .....	1
Variability of the Hering-Breuer Reflexes in the Dog Under Sodium Evipal Anesthesia. <i>Carl A. Moyer and Henry K. Beecher</i> .....	7
Central Stimulation of Respiration During Hypoxia. <i>Carl A. Moyer and Henry K. Beecher</i> .....	13
The Influence of the Pituitary and Adrenal Cortex on Resistance to Low Environmental Temperatures. <i>R. Tyslowitz and E. B. Astwood</i> .....	22
Experimental Investigation on the Effects of Trauma and Traumatic Shock on Gastrointestinal Motility and Secretions. <i>H. Necheles and Wm. H. Olson</i> .....	32
The Effect of Denervation on the Filtration Rate and Blood Flow in Dog Kidneys Rendered Hyperemic by Administration of Pyrogen. <i>Edwin P. Hiatt</i> .....	38
Water Intoxication of the Frog ( <i>Rana pipiens</i> ). <i>Edward C. Schneider and Wilson C. Grant</i> .....	42
The Respiration of Neurones. <i>Jane Pearce and R. W. Gerard</i> .....	49
Oxidases, Pressor Amines and Hypertension. <i>Gustav J. Martin, C. T. Ichniowski, W. A. Wisansky and S. Ansbacher</i> .....	66
Studies of the Metabolism of Bovine Epididymal Spermatozoa. <i>Gertrude Henle and Charles A. Zittle</i> .....	70
The Use of Double Work Periods in the Study of Fatigue and the Influence of Caffeine on Recovery. <i>Eliot Foltz, A. C. Ivy and C. J. Barborka</i> .....	79
The Circulatory Response of the Unanesthized Dog to Small Physiological Quantities of Adrenalin. <i>Harold C. Wiggers, Alfred M. Duschatko and Ross C. Kory</i> .....	87
The Anterior Pituitary in the Carbohydrate Metabolism of the Eviscerated Rat. <i>Jane A. Russell</i> .....	95
Heat Exchanges During Recovery from Experimental Deficit of Body Heat. <i>E. A. Pinson and E. F. Adolph</i> .....	105
Pressure of Blood in the Right Auricle in Animals and in Man: Under Normal Conditions and in Right Heart Failure. <i>Dickinson W. Richards, Jr., Andre Cournand, Robert C. Darling, William H. Gillespie and Eleanor DeF. Baldwin</i> .....	115
The Interrelationship of p-Aminobenzoic Acid and Inositol. <i>Gustav J. Martin</i> .....	124
Influence of Anterior Pituitary Extract on Protein and Carbohydrate Metabolism. <i>Karl E. Paschkis</i> .....	128
The Influence of Diethyl-Stilbestrol on Carbohydrate Metabolism in Normal and Castrated Rats. <i>R. G. Janes and W. O. Nelson</i> .....	136
Observations on the Physiological Reactions of the Ductus Arteriosus. <i>J. Allen Kennedy and Sam L. Clark</i> .....	140
Training and Its Effects on Man at Rest and at Work. <i>C. A. Knehr, D. B. Dill and William Neufeld</i> .....	148
The Reaction and Neutralizing Ability of the Contents of the Pyloric Antrum and First Part of the Duodenum in Normal Dogs Following an Ewald Meal. <i>J. Edward Berk, J. Earl Thomas and Martin E. Rehfsuss</i> .....	157
The Metabolism of Fructose by the Eviscerated Rat. <i>Roger M. Reinecke</i> .....	167
The Effect of the Urethane of Beta Methylcholine Chloride upon the Parasympathectomized Cat's Eye. <i>Francis C. Keil, Jr. and Walter S. Root</i> .....	173
The Changes Produced on the Oxygen and Carbon Dioxide Content of Arterial and Venous Blood of the Brain during Diathermy Therapy for General Paresis. <i>Joseph M. Looney and Embric J. Borkovic</i> .....	177

An Analysis of the Action of Acetylcholine on the Cardiac Ganglion of <i>Limulus polyphemus</i> . <i>W. E. Garrey</i> .....	182
The Effect of Hypertonic Plasma on the Body Fluids in Normal Experimental Animals. <i>C. T. Ashworth, E. E. Muirhead and J. M. Hill</i> .....	194
Combination of Hypoxic and Hypercapnic Stimulation at the Carotid Body. <i>Claude V. Winder</i> .....	200
The Flow of Lymph from the Lungs of the Dog. <i>Madeleine F. Warren and Cecil K. Drinker</i> .....	207

## No. 2. APRIL, 1942

Ciliary Movement and Circulation of Cerebrospinal Fluid within Brain Ventricles in Larval and Adult Anurans. <i>Hsiang-Yao Chu</i> .....	223
Urine Dilution and Concentration Tests in Adrenalectomized Dogs. <i>Frederic J. Kottke, Charles F. Code and Earl H. Wood</i> .....	229
Absorption and Disposition of Glucose in the Chick. <i>W. R. C. Golden and C. N. H. Long</i> .....	244
Observations on the Accuracy of the Thermostromuhr. <i>D. E. Gregg, W. H. Pritchard, R. W. Eckstein, R. E. Shipley, A. Rotta, J. Dingle, T. W. Steege and J. T. Wearn</i> ..	250
Operative Mechanism of Some Errors in the Application of the Thermostromuhr Method to the Measurement of Blood Flow. <i>R. E. Shipley, D. E. Gregg and J. T. Wearn</i> .....	263
The Homeostatic Rôle of a Renal Humoral Mechanism in Hemorrhage and Shock. <i>Angie S. Hamilton and Dean A. Collins</i> .....	275
The Effect of a Cream Meal on the Acidity and Neutralizing Ability of the Contents of the Duodenal Bulb in Normal Dogs. <i>J. Edward Berk, J. Earl Thomas and Martin E. Rehfuess</i> .....	285
Precocious Gonadal Development Occurring in Immature Rats Following a Short-Time Treatment with Antigonadotropic Serum. <i>Herbert S. Kupperman, Roland K. Meyer and John C. Finerty</i> .....	293
Plasma Protein Replacement after Hemorrhage in Dogs With and Without Shock. <i>Richard V. Ebert, Eugene A. Stead, Jr., James V. Warren and William E. Watts</i> ....	299
The Relation of the Pituitary, Thyroid and Adrenal Glands to the Maintenance of Normal Serum Albumin and Globulin Levels. <i>Louis Levin and James H. Leatham</i> ...	306
Linear Relationship Between the Circulating Red Cell Mass and the Venous Hematocrit as Determined with Radioactive Iron. <i>P. F. Hahn and W. F. Bale</i> .....	314
Idioventricular Rhythms and Fibrillation Induced at the Anode or the Cathode by Direct Currents of Long Duration. <i>A. Sidney Harris and Gordon K. Moe</i> .....	318
The Action of Ions on the Mammalian Heart. <i>C. R. Spealman</i> .....	332
The Influence of Bile Salts on Active Intestinal Absorption of Chloride. <i>H. C. Peters</i> . 340	
The Effect of Low Potassium Diet and of Desoxycorticosterone Acetate upon Renal Size. <i>Stanley H. Durlacher, Daniel C. Darrow and Milton C. Winternitz</i> .....	346

## No. 3. MAY, 1942

Ion Antagonism and the Frog Heart. <i>C. R. Spealman</i> .....	351
A Defect in the Coagulation Mechanism of Swine Blood. <i>M. E. Muhrer, A. G. Hogan and R. Bogart</i> .....	355
The Anomaly of a Normal Duke's and a Very Prolonged Saline Bleeding Time in Swine Suffering from an Inherited Bleeding Disease. <i>Edwin T. Mertz</i> .....	360
The Effect of Body Size Upon Energy Exchange in Work. <i>Sid Robinson</i> .....	363
The Reaction and Neutralizing Ability of the Contents of the First Part of the Duodenum in Normal Dogs Under Fasting Conditions. <i>J. Edward Berk, J. Earl Thomas and Martin E. Rehfuess</i> .....	369
Hypothalamic Stimulation Yielding Adrenalin Reversal Effects. <i>M. B. Bender and E. A. Weinstein</i> .....	376

The Influence of Posture on Blood Flow in the Dog. <i>H. S. Mayerson</i> .....	381
Effect of Fasting and Feeding on the Response of Surviving Tissues to Quinidine. <i>Fred G. Brazda and James C. Rice</i> .....	386
The Influence of Benzedrine on Work-Decrement and Patellar Reflex. <i>Gordon A. Alles and George A. Feigen</i> .....	392
Observations on Hemorrhagic Hypotension and Hemorrhagic Shock. <i>J. M. Werle, R. S. Cosby and C. J. Wiggers</i> .....	401
Cardiac and Peripheral Resistance Factors as Determinants of Circulatory Failure in Hemorrhagic Shock. <i>C. J. Wiggers and Jacob M. Werle</i> . With technical assistance of <i>P. C. Shea</i> .....	421
A Study by Quantitative Methods of the Spontaneous Variations in Volume of the Finger Tip, Toe Tip, and Postero-Superior Portion of the Pinna of Resting Normal White Adults. <i>G. E. Burch, A. E. Cohn and C. Neumann</i> .....	433
A Study of the Relationship Between the Pulse and Alpha Waves of the Tips of the Fingers and Toes of Five Adults. <i>C. Neumann, A. E. Cohn and G. E. Burch</i> .....	448
A Study by Quantitative Methods of the Spontaneous Variations in Volume of the Tips of the Fingers and Toes and Postero-Superior Portion of the Pinna of Hypertensive Patients and Senile Subjects. <i>C. Neumann, A. E. Cohn and G. E. Burch</i> .....	451
The Disappearance Rate of Tyrosine from the Blood of Dogs. <i>James A. Greene and George W. Johnston</i> .....	460
The Absolute Threshold of Vision in Cat and Man with Observations on Its Relation to the Optic Cortex. <i>Charles S. Bridgman and Karl U. Smith</i> .....	463
Chemical Composition of Human Semen and of the Secretions of the Prostate and Seminal Vesicles. <i>Charles Huggins, William W. Scott and J. Henry Heinen</i> .....	467
Histochemical Changes in the Myocardium of Dogs Following Experimental Temporary Coronary Arterial Occlusion. <i>Oliver H. Lowry, D. Rourke Gilligan and A. Baird Hastings</i> .....	474
Some Special Instances of Predominant Chemical Stimulation of the Expiratory Half-Center. <i>Robert Gesell, Carl A. Moyer and John B. McKittrick</i> .....	486
The Effect of Sulfanilamide on the Ability of Rabbits and Dogs to Withstand High Altitudes. <i>Frank Lawrence Lawson</i> .....	494
The Measurement of Edema in the Heart-Lung Preparation. <i>Earl H. Wood and Gordon K. Moe</i> .....	506
Electrolyte and Water Content of the Ventricular Musculature of the Heart-Lung Preparation with Special Reference to the Effects of Cardiac Glycosides. <i>Earl H. Wood and Gordon K. Moe</i> .....	515
The Normal Pneumocardiogram. <i>H. A. Blair, A. M. Wedd and H. M. Hardwicke</i> ....	523
Titration of Human Seminal Fluid with Acids and Alkalis and Their Effects on the Survival of Sperm Motility. <i>Leo Shedlovsky, Donald Belcher and Irving Levenstein</i> .	535

## NO. 4. JUNE, 1942

The Activity of the Pacemaker Previous to the Discharge of a Muscular Impulse. <i>Emil Bozler</i> .....	543
The Action Potentials Accompanying Conducted Responses in Visceral Smooth Muscles. <i>Emil Bozler</i> .....	553
The Activity of the Descending Duodenum During Nausea. <i>Franz J. Ingelfinger and Robert E. Moss</i> .....	561
Differences Among Adrenal Steroids with Respect to Their Efficacy in Protecting the Adrenalectomized Dog Against Circulatory Failure. <i>W. W. Swingle, J. W. Remington, V. A. Drill and W. Kleinberg</i> .....	567
The Effect of Depletion of Body Potassium on the Time of Survival After Nephrectomy and Ureteral Ligation. <i>Stanley H. Durlacher and Daniel C. Darrow</i> .....	577
Effects of the Removal of the Anterior Lobe of the Hypophysis on Some Renal Functions. <i>H. L. White, Peter Heinbecker and Doris Rolf</i> .....	584

Relation of the Adeno and the Neurohypophysis to Insulin Sensitivity and Sugar Tolerance in the Dog. <i>Peter Heinbecker, H. L. White and Doris Rolf</i> .....	592
Production of Insulin Resistance in Depancreatized Dogs. <i>James A. Greene, Ann David and George Johnston</i> .....	595
The Respiration of the Developing Brain. <i>D. B. Tyler and A. van Harreveld</i> .....	600
An Acid-Neurohumoral Mechanism of Nerve Cell Activation. <i>Robert Gesell, Charles R. Brassfield and Mary Alice Hamilton</i> .....	604
The Spontaneous Activity and Food Intake of Rats with Hypothalamic Lesions. <i>A. W. Hetherington and S. W. Ranson</i> .....	609
Muscle Tremors and the Development of Temperature Regulation in Birds. <i>Eugene P. Odum</i> .....	618
Speed of Responses of Various Muscles of Cats. <i>J. H. Wills</i> .....	623
Accommodation in Mammalian Motor Nerves. <i>A. Rosenblueth and E. C. del Pozo</i> ....	629
Some Actions of Indole on the Dog. <i>Harold N. Ets and I. M. Feinberg</i> .....	647
The Etiology of Hypericism, a Photosensitivity Produced by St. Johnswort. <i>Nello Pace</i>	650
Water Metabolism of the Chicken ( <i>Gallus domesticus</i> ) with Special Reference to the Rôle of the Cloaca. <i>William M. Hart and Hiram E. Essex</i> .....	657
The Vasomotor Components in the Vascular Reactions in the Finger to Cold. <i>Alrick B. Hertzman and Laurence W. Roth</i> .....	669
The Reactions of the Digital Artery and Minute Pad Arteries to Local Cold. <i>Alrick B. Hertzman and Laurence W. Roth</i> .....	680
The Absence of Vasoconstrictor Reflexes in the Forehead Circulation. Effects of Cold. <i>Alrick B. Hertzman and Laurence W. Roth</i> .....	692

## No. 5. JULY, 1942

The Effects of Veratrine upon the Superior Cervical Ganglion. <i>A. Rosenblueth and E. C. del Pozo</i> .....	699
The Effect of Adrenalectomy on the Absorption of Hydrogenated Cottonseed Oil, Corn Oil, Tributyrin and Sodium Butyrate. <i>Lucien A. Bavetta and Harry J. Deuel, Jr.</i> .....	712
The Parathyroids and the Clearance of Inorganic Phosphate. <i>Marion Fay, Vivian G. Behrmann and Dorothy M. Buck</i> .....	716
The Salivatory Motor Nuclei in the Monkey. <i>H. W. Magoun and Lindsay E. Beaton</i> ..	720
The Nature of the S Complex of the Electrocardiogram. <i>L. H. Nahum, H. E. Hoff and W. Kaufman</i> .....	726
Specificity in the Renin-Hypertensinogen Reaction. <i>John W. Bean</i> .....	731
Muscle Potentials Accompanying a Single Volitional Twitch. <i>J. E. P. Toman and Robert H. Oster</i> .....	743
The Effect of Ether and Starvation on Liver Glycogen Maintenance after Various Diets. <i>Robert A. Newburger and Frederick R. Brown</i> .....	746
Transfer of Water Across the Placenta of the Guinea Pig. <i>Alfred Gellhorn and Louis B. Flexner</i> .....	750
The Transfer of Water and Sodium to the Amniotic Fluid of the Guinea Pig. <i>Louis B. Flexner and Alfred Gellhorn</i> .....	757
Studies on the Relation of the Liver Function, Pulse Rate and Temperature of Hyperthroid Dogs to Vitamin B <sub>1</sub> and Yeast. <i>Victor A. Drill and Harry W. Hays</i> ..	762
Vanadium—A Consideration of Its Possible Biological Rôle. <i>Esther Peterson Daniel and Elizabeth M. Hewston</i> .....	772
The Effects of Adrenalectomy and Replacement Therapy on the Serum Protein Levels of the Cat. <i>Louis Levin, J. H. Leatham and R. C. Crafts</i> .....	776
Studies on Irradiated Cerebral Differentiated Excitation and Inhibition as Indicated and Measured by Respiration. <i>William F. Allen</i> .....	783
Index.....	797





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No. 1

## THE EFFECT OF HYPOGLYCEMIA ON THE ELECTROENCEPHALOGRAM AT VARYING DEGREES OF OXYGENATION OF THE BLOOD

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The interaction of anoxia and hypoglycemia has been the subject of a number of studies (E. Gellhorn and collaborators, McQuarrie and others). It has been shown that the vasomotor center is greatly sensitized to anoxia at low blood sugar levels (Gellhorn, Ingraham and Moldavsky). On the other hand, hypoglycemic convulsions were inhibited rather than enhanced by anoxia (McQuarrie, Gellhorn, Packer and Feldman.) It therefore seemed to be of interest to investigate whether a synergistic action of anoxia and hypoglycemia exists with regard to the somatic nervous system if criteria other than convulsions are chosen as indicators. For this purpose the effect of hypoglycemia on the electroencephalogram was studied at varying degrees of oxygenation of the blood.

The electroencephalogram (E.E.G.) was chosen as an indicator of cortical somatic excitability although it is well known that the cortex contains efferent neurons influencing the activity of autonomic centers at lower levels. However, it is well established that conditions involving a decreased excitability of the cortical somatic centers are paralleled by corresponding changes in the E.E.G.

**METHOD.** The experiments were performed on anesthetized cats (100 mgm./kgm. chloralose subcutaneously) and on unanesthetized rats. In the former blood pressure was recorded from the carotid artery with the Hg manometer and the brain potentials were led off by means of wick electrodes from the parietal lobe of the brain or from the skull by inserting two screws into the calvarium. In the rat experiments we employed Hoagland's technique and inserted two phonograph needles, insulated except for the tip, into the skull of the rats. For recording the potentials a push-pull amplifier and an Offner ink recorder were used.

The cats inhaled oxygen/nitrogen mixtures from Douglas bags; the rats were placed in suitable bottles through which the desired oxygen/nitrogen mixtures were blown. In order to secure regularly hypoglycemia adreno-demedullated

<sup>1</sup> Aided by a grant from the John and Mary R. Markle Foundation.



rats were used. Five units of insulin<sup>2</sup> per kilogram were injected subcutaneously.

**RESULTS** 1. *Interaction of anoxia and hypoglycemia.* If rats are allowed to inhale 7 per cent oxygen no conspicuous changes in the E.E.G. are observed when the animals are subjected to this low oxygen tension for an hour or more. If, however, 7 per cent oxygen is administered during insulin hypoglycemia profound alterations in the E.E.G. occur.

Figure 1 shows that inhalation of 7 per cent oxygen changes the electrical potentials fundamentally by causing very large, slow potentials to appear on which the fast alpha waves are superimposed. On readmission of air a gradual restoration of the original alpha waves is observed. If, then, glucose is injected and the hypoglycemia thereby eliminated the inhalation of 7 per cent oxygen, even for a longer period of time, remains without effect.

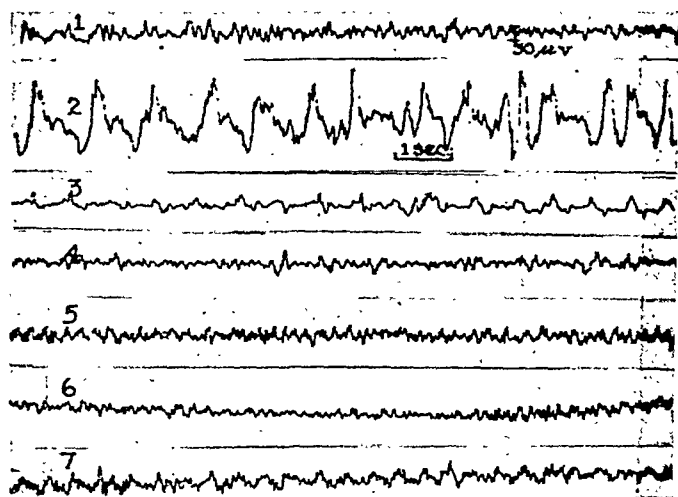


Fig. 1. Adrenalectomized rat. Five U. insulin subcutaneously. 1, 21 min. after injection. 2, 2 min. 7 per cent O<sub>2</sub>. 3, after 2 min. in air, 4, after 3 min. in air; blood sugar 43 mgm. per cent; 7 cc. 10 per cent glucose intraperitoneally. 5, 25 min. later (air) 6, 3 min. 7 per cent O<sub>2</sub>. 7, 8 min. after air.

It is very interesting to note that the type of effect illustrated in figure 1 is similar to that occurring without the addition of anoxia in insulin hypoglycemia, provided that the blood sugar falls sufficiently low and this condition is maintained for an adequate length of time. This and similar observations suggest that anoxia and hypoglycemia act synergistically on the cortical potentials.

Whereas figure 1 illustrates the action of anoxia on the brain potentials at a relatively mild hypoglycemic state the effects resulting from the interaction of anoxia and hypoglycemia when the blood sugar has fallen to a very low level (30 mgm. per cent) are slightly different. Under these conditions the alpha waves are greatly diminished in amplitude and slow delta waves appear. On administration of 7 per cent oxygen both alpha and delta waves are diminished so that the brain potentials disappear almost completely. On readmission of air brain waves similar to those obtained prior to the administration of 7 per cent oxygen are gradually restored.

<sup>2</sup> Kindly supplied by Eli Lilly & Company.

Figure 2 shows a record from an experiment on an anesthetized cat in which not only the effect of anoxia at various blood sugar levels on the E.E.G. but also on the blood pressure is recorded. Although hypoglycemia does not alter the E.E.G. of the cat in chloralose anesthesia to as great a degree as seen in the unanesthetized rat the reaction to anoxia is basically the same. In the anesthetized cat, too, it is seen that anoxia and hypoglycemia act synergistically on the brain potentials and that the effect on the E.E.G. resulting from anoxia increases progressively with falling blood sugar. No effects are seen after inhalation of 8.1 per cent oxygen at a blood sugar level of 108 mgm. per cent,

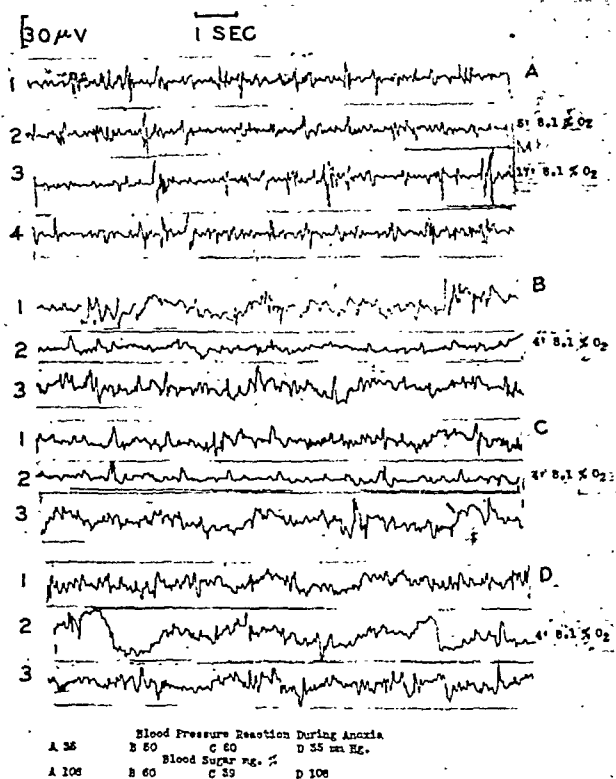


Fig. 2. Cat, 100 mgm. chloralose per kilogram subcutaneously; 5 U. insulin. First and last record in A, B, C and D is obtained while the animal is inhaling air. Second and third in A and second in B, C and D were obtained during inhalation 8.1 per cent  $O_2$ .

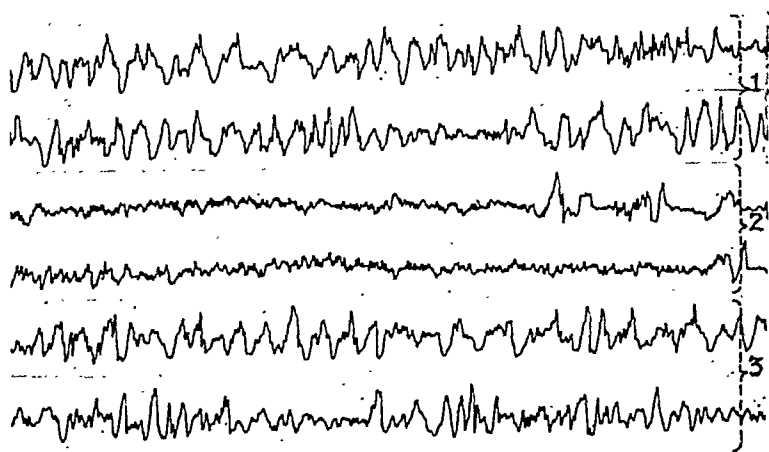
Blood pressure reaction during anoxia in mm. Hg: A, 35; B, 50; C, 60; D, 35.

Blood sugar, mgm. per cent: A, 108; B, 60; C, 39; D, 108.

even after seventeen minutes, whereas at 60 mgm. per cent and still more at 39 mgm. per cent blood sugar a profound diminution of the potentials occurs. The fundamental difference in the reactivity of the autonomic and somatic systems is well illustrated in this experiment. The blood pressure rises in response to anoxia to an increasing degree with progressing hypoglycemia whereas the cortical potentials are diminished progressively under the same conditions. The synergism of anoxia and hypoglycemia is present for both autonomic and somatic nervous systems, but whereas the somatic system suffers progressively in its

excitability under these conditions, the autonomic nervous system shows a greatly increased reactivity.

2. *The effect of inhalation of pure oxygen on the E.E.G. under conditions of hypoglycemia.* The fact illustrated in the preceding paragraphs, that the effects of low oxygen on the E.E.G. are greatly enhanced under conditions of hypoglycemia, made it not improbable that the effects of hypoglycemia may be alleviated to a certain extent by improving the degree of oxygen saturation of the blood and consequently of the brain. We therefore attempted to show that the effects of hypoglycemia on the E.E.G. could be diminished by the inhalation of pure oxygen. These experiments were carried out on unanesthetized rats exclusively. Although the inhalation of 100 per cent oxygen has no effect on the E.E.G. of a normal rat, it produced marked changes under conditions of insulin hypoglycemia.



Adrenalectomized rat. 5 u. insulin subcut.  
Time after injection:- 1- 81 min. in air ;  
2- after  $4\frac{1}{2}$  min. in 100%  $O_2$  ; 3- 5 min. after air

Fig. 3. Adrenalectomized rat. Five U. insulin subcutaneously. Time after injection: 1, 81 min. in air. 2, after  $4\frac{1}{2}$  min. in 100 per cent  $O_2$ ; 3, 5 min. after air.

Figure 3 shows a record in which the injection of insulin had produced large delta waves on which the more frequent alpha waves were superimposed. This typical effect of hypoglycemia occurred 81 minutes after the injection of insulin into the adreno-demedullated rat. When the animal was allowed to inhale oxygen and a record was taken after  $4\frac{1}{2}$  minutes of oxygen inhalation, the delta waves had largely disappeared and a record was obtained similar to that found prior to the injection of insulin. If, however, oxygen was no longer administered and the animal inhaled air the delta waves reappeared and the record was practically identical with that obtained prior to the administration of pure oxygen.

It is frequently found that the E.E.G. during hypoglycemia consists of periods of delta waves with alpha potentials superimposed on them alternating with periods of alpha potentials exclusively. Also in this case the inhalation of 100 per cent oxygen abolished the delta potentials thereby restoring an E.E.G. of pure alpha potentials as existed before the administration of insulin.

The experiments show clearly that the effects of hypoglycemia can be completely offset by the inhalation of pure oxygen. It is, however, important to note that this statement does not apply for all conditions of hypoglycemia. If the hypoglycemia is so severe and prolonged as to lead to an almost complete disappearance of brain potentials (prior to the convulsive state) no restoration of normal brain potentials results from the administration of oxygen under these conditions, although brain potentials are usually restored by the injection of sugar.

**DISCUSSION.** In several investigations on anoxia (Cortell, Carlson, Greenberg, Lambert and Gellhorn), it was shown that whereas the somatic nervous system is depressed during anoxia, the sympathetic system is in a state of greater tonicity and excitability. It is interesting to note that this difference in the reactivity existing between the somatic and autonomic nervous system is preserved under conditions involving anoxia and hypoglycemia at the same time. It was shown by the fact that hypoglycemia and anoxia depressed cortical potentials more than corresponds to the algebraic summation of the effects produced by anoxia and hypoglycemia when studied separately. A similar synergism exists with regard to the autonomic nervous system. When the reactivity of the vasomotor center is studied it is seen in a greatly increased rise in blood pressure under conditions of anoxia and hypoglycemia. The synergism seen on the autonomic nervous system may be interpreted as an attempt to offset the synergism acting on the somatic nervous system for the purposes of homeostasis.

Our experiments give further support to the assumption that the rate of oxidation of the brain depends on the sugar level as well as on the oxygen tension of the blood (cf. Gellhorn, Ingraham and Moldavsky concerning the older literature). This assumption makes it understandable that the effects of anoxia on the E.E.G. are aggravated by the hypoglycemia, and the changes in the brain potentials induced by hypoglycemia can be offset by the inhalation of 100 per cent oxygen. There are, however, definite limits to the possibility of substituting glucose for oxygen. Although injection of glucose restores brain function when its activity is almost completely lost due to hypoglycemic coma, no such effects are obtained with pure oxygen inhalation; but the latter procedure is effective as long as the hypoglycemia has not proceeded further than to the state in which the brain potentials are characterized by the partial or exclusive occurrence of large delta waves. Moreover, no clear evidence could be found that an increase in blood sugar above the normal level resulting from injection of glucose would increase the resistance of the brain to anoxia.

The observations reported in this paper support the interpretation which McFarland and Forbes give to their work on the effect of hypoglycemia and anoxia on visual dark adaptation, namely, that the synergistic effects of anoxia and hypoglycemia as well as the antagonistic effects of pure oxygen on the effect of hypoglycemia are not due to their action on the visual purple but on that part of the central nervous system which is involved in vision.

The experiments cannot be explained on the basis of alterations in the blood sugar induced by the inhalation of 7 per cent oxygen since in order to produce a significant lowering of the blood sugar level in adrenalectomized rats two hours of anoxia are required but not 2 to 3 minutes as used in the present experiments. Moreover, the synergism of anoxia and hypoglycemia on brain potentials was present in adrenalectomized as well as adrenalectomized-vagotomized animals although even prolonged anoxia failed to alter the blood sugar in the latter group (Feldman, Cortell and Gellhorn).

#### SUMMARY

The interaction of hypoglycemia and anoxia, and the effect of the inhalation of pure oxygen during insulin hypoglycemia was studied in anesthetized cats and unanesthetized rats with regard to brain potentials. It was found that: 1, the effect of anoxia on brain potentials is greatly aggravated during insulin hypoglycemia; 2, the action of hypoglycemia on the brain potentials can be offset by the inhalation of pure oxygen.

The experiments support the assumption that the excitability of the cortex in vivo depends on both oxygen tension and blood sugar, and that both factors are involved in the rate of oxidations of the brain.

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# VARIABILITY OF THE HERING-BREUER REFLEXES IN THE DOG UNDER SODIUM EVIPAL ANESTHESIA<sup>1</sup>

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The changes in the character of the respiratory act associated with inflation and deflation of the lungs are considered as being due to the interaction of inhibition and excitation. The specific inhibitory as well as excitatory functions of the pulmonary afferent impulses have been generally accepted though variously interpreted. (For a concise review of past and current concepts regarding the Hering-Breuer phenomena, see (2) or (3).)

Gross alterations in the Hering-Breuer reflexes associated with changes in depth of sodium evipal anesthesia have been observed and are considered pertinent to a better understanding of these phenomena. The purpose of this paper is to illustrate these changes and their significance.

**METHODS.** The experimental methods are the same as previously described (5). Inflation and deflation of the lungs were carried out with the chest wall intact, by increasing and decreasing the air pressure within the respiration system to which the animals were connected. All pressures employed were 70 mm. of water above or below atmospheric pressure. Seventy millimeters water pressure above atmospheric is described as "positive" or inflationary; 70 mm. water below atmospheric pressure is for convenience described as "negative" or deflationary. This study is based upon experiments upon 21 dogs.

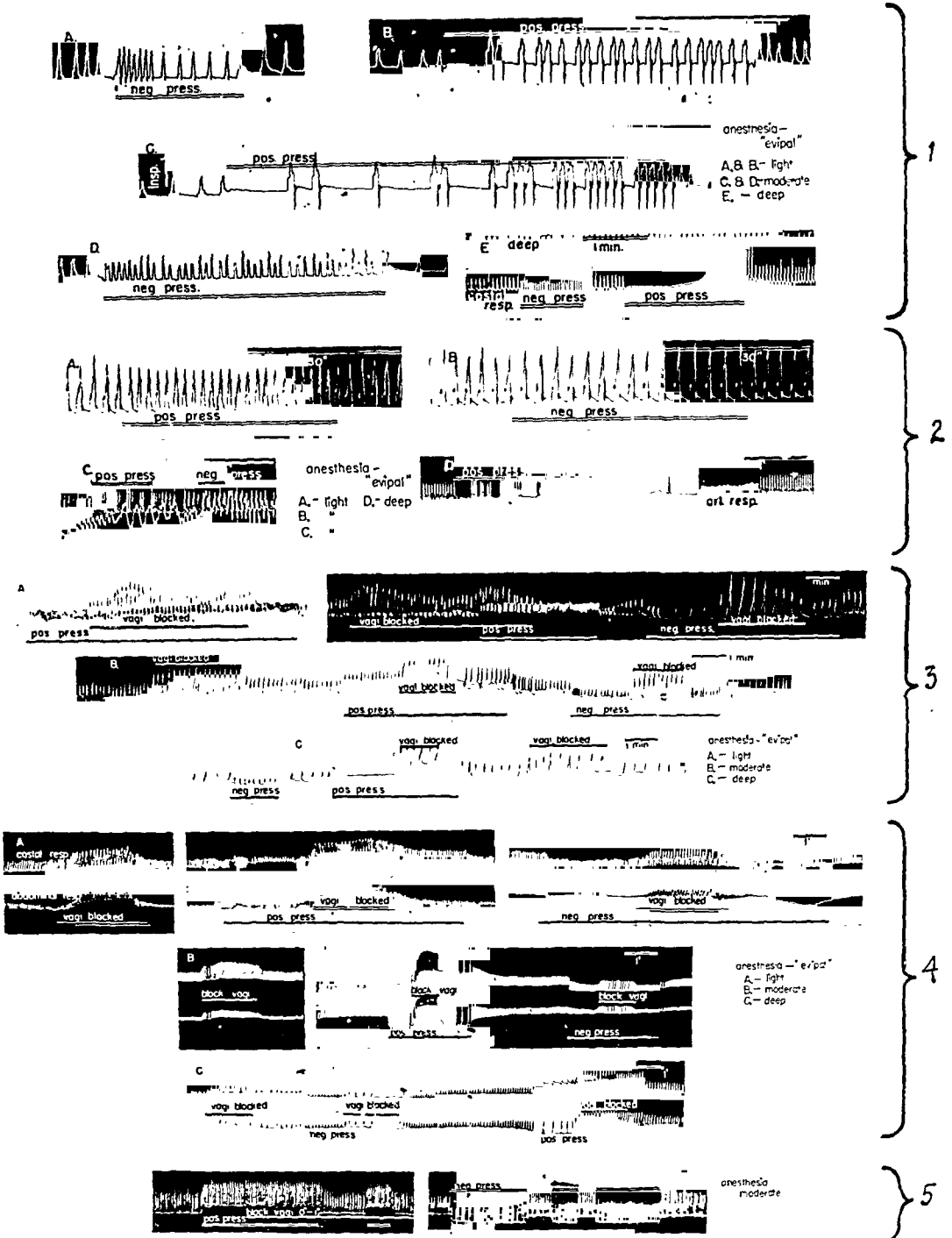
**RESULTS.** Figure 1: a reduction in lung volume (neg. press.) in this lightly anesthetized animal induced an initial quickening of breathing (the typical response) that lasted for seven breaths. The rate then became less than the antecedent normal, probably due to a decrease in central chemical drive (CO<sub>2</sub>) (5). After the anesthesia was deepened slightly (D) the same deflation produced an identical initial acceleration which did not diminish appreciably. Under relatively deep anesthesia (E) the effectiveness of pulmonary deflation in speeding breathing was great.

Under light anesthesia a transient slowing of respiration followed lung inflation. The breathing during the inflation was characterized by regular, rapid, forceful expirations; these forceful expirations were much smaller and intermittent before inflation.

After deepening the anesthesia (C) the active expirations are brought on by

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Figs. 1, 2, 3, 4 and 5. Read from left to right. An upstroke indicates inspiration. Pos. press., neg. press. indicate respectively inflation and deflation pressures of 70 mm. H<sub>2</sub>O. greater or less, respectively, than atmospheric pressure.

inflation although entirely absent under normal lung volume. The slowing phase of inflation is much longer, paralleling decreasing sensitivity to carbon

dioxide, and the terminal acceleration is much more in evidence. The rate in this latter phase is greater than that which obtains following removal of the positive pressure (C).

Figure 2 illustrates a type response to inflation and deflation frequently seen under *light* evipal (and pentothal) anesthesia. Lung inflation augments breathing by shortening the duration of inspiration and expiration; lung deflation slows it by lengthening both phases. After the anesthesia was deepened (C and D) lung inflation (pos. press.) which previously shortened expiration now prolonged it.

The responses of the animal shown in figure 3 are somewhat comparable to those of figure 2. Inflation accelerates breathing and deflation slows it. Vagal block during inflation (A) does not slow breathing as much as it does when lung volume is normal; vagal block during negative pressure is associated with a greater increase in amplitude and a slower rate than obtains with normal and greater than normal lung volumes. After more sodium evipal is given (B) the speeding with inflation is less and deflation augments breathing instead of slowing it. The differences in reaction to vagal cold block observed under light anesthesia with changing lung volume are no longer apparent. The administration of more anesthetic changes the inflation response to a slowing of breathing that is abolished by vagal block; vagal block formerly merely decreased the acceleration. Deflation still speeds respiration.

Another type variation is illustrated in figure 4. When very lightly anesthetized the animal reacted to lung inflation and deflation by a minor slowing and a fair augmentation of breathing. Vagal block during inflation produced further slowing and abolished the speeding of deflation. The short periods of initial and terminal stimulation are most likely of general somatic afferent origin; the stimuli are provided by the starting and stopping of the experimental procedures. Under moderate anesthesia (B) the rate of breathing with normal innervation and with the vagi blocked was faster than the breathing under similar circumstances with lighter anesthesia (A). Pulmonary inflation produced a prolonged powerful expiration especially abdominally (B); blocking the vagi did away with the powerful expiratory effort shown by the rapid inspiratory progression of both costal and abdominal records which immediately followed the application of the blocks. Deblocking reinstituted the expiratory effort. Vagal block during the fast breathing of decreased lung volume was associated with a slower breathing than was present during block under normal and supernormal lung volumes. The same relationship of rates under block during normal and decreased lung volume are noted in (C) under deep anesthesia. The rate under block and inflation in (C) was greater than that during block with lung volume normal. The slowing and the increased strength of expiration that attends inflation was less when the anesthesia was deep than when moderate.

Figure 5 illustrates an atypical reaction to inflation and deflation that was unchanged by block and did not become typical at any depth of anesthesia short of death.

DISCUSSION. While certain variants are presented in the figures during light



anesthesia, the reactions of *deeply* anesthetized animals to pulmonary inflation and deflation are almost invariably qualitatively similar and are of this type: inflation slows and deflation speeds breathing. Under light sodium evipal anesthesia great variability is noted. If the reactions typically found under deep anesthesia are present under light anesthesia they are of lesser degree and are less well maintained than under deeper anesthesia. Compare A and B with E of figure 1, and A with B and C of figure 4. Frequently lung inflation speeds and deflation slows breathing with light anesthesia and yet produces the usual responses with deep. Compare A and B with C and D of figure 2, and A with C of figure 3. Occasionally when light the animal will initially react with the typical deep anesthesia response and shift during the procedure to the opposite reaction type as in A, B and C, of figure 1. Those animals that react to an increased and decreased lung volume with an increased and decreased rate of breathing respectively fall into two main groups: those in which vagal block diminished the response, figure 3 A, and those in which it does not, figure 5. The reaction of the former group is therefore at least in part due to pulmonary vagal afferent stimulation and that of the latter mainly if not entirely due to extra vagal stimuli. The source of the extravagal excitation has not been determined; it is presumed that it lies in the respiratory muscle and joint proprioceptors (1, 6). Increasing the depth of anesthesia obliterated the counter-typical reactions in five cases out of the six observed; the one exception is the experiment from which figure 5 was taken. Figures 2 and 3 illustrate the transition effected in the major group.

The above variations of response and their transitions cannot be tentatively explained upon the main premise that a changing inhibition of breathing is the predominant action of the vago-pulmonary afferent stimulation associated with lung volume change. However, if one should employ as a main premise the tenets of pulmonary vagal reflex action held by Gesell (3), a logical explanation may be constructed very simply. In Gesell's schema afferent impulses of pulmonary-vagal origin are predominantly excitatory and inhibition is considered as a reciprocal function of the primary respiratory motor neurones and not as an attribute of the vagal afferent system. Whether the stimuli of pulmonary origin will be reflected as increased inspiratory or expiratory activity will depend upon the precedence that the stimulus is able to command. Precedence is determined largely by the strength and character of the stimulus, the peculiarities of the central connections of the afferent pathway over which the impulses are propagated, and the balance of the prevailing inspiratory and expiratory drives before arrival of the stimulus.

In these experiments the strength of stimulus is assumed to be constant throughout a single experiment, a constant inflating and deflating force being used. The peculiarities of the central connections of the vagal afferent fibers stimulated are also considered as a constant in any one animal upon anatomical grounds. Impulses set off by inflation stimulate inspiration and expiration, expiration to the greatest extent. The fibers carrying these impulses are therefore thought to have more abundant connections with the expiratory center.

The converse relationship holds for the deflation reflex; thus its receptors may be considered to have more numerous inspiratory central connections. Therefore the variations in the Hering-Breuer reflexes that follow changes in the depth of anesthesia are most likely in greatest part due to change in the balance of the prevailing drives prior to and during the stimulation.

One major change in respiratory drive is now known to take place as the anesthesia produced by sodium evipal is deepened; the central chemical drive, as measured by the animal's response to a fixed increase in the carbon dioxide of the inspired air, is very much more rapidly and profoundly depressed than are the reflex drives, chemical and proprioceptive (5). Since increased central acidity ( $\text{CO}_2$ ) is predominantly an inspiratory drive (3) increasing the depth of the sodium evipal anesthesia will increase the apparent effectiveness of any reflex expiratory drive such as the pulmonary vagal inflation reflex. This is considered to be the probable reason for the change in reaction to pulmonary inflation and deflation that takes place in figures 1 and 4 as the anesthesia is deepened.

Another disturbance in drive balance depends upon the manner of producing the pulmonary vagal stimulation. In this series of experiments, since the chest wall is intact, inflation and deflation of the lungs stimulate both vagal and extravagal afferent systems. The effect upon breathing produced by this extravagal stimulation is, in this series of experiments, opposite to that seen on solely inflating and deflating the lungs; the expansion of the chest and the decreasing diaphragmatic tension attending lung inflation increases the frequency of breathing, and deflation of the chest and increasing diaphragmatic tension slows breathing. Figure 5 illustrates practically a total "extravagal reflex" action in that vagal block has very little effect upon the reactions to positive and negative pressure. Individual variations in the balance between the extravagal and pulmonary vagal proprioceptive drives are seemingly more or less responsible for the differences in reaction to lung volume change at a given level of anesthesia. For example: if the extravagal and pulmonary vagal drives produced by a lung volume change should be equal then the visible effect upon breathing will be slight if any as in A of figure 4; if the extravagal drive is the stronger then the rate of breathing would increase with lung expansion and slow with deflation, figures 2 A, B, 3 A, and 5; should the vagal drive exceed the extravagal the classical Hering-Breuer reflex responses would be obtained as in A, figure 1. If deepening the anesthesia depresses the extravagal drive more than the vagal then an animal having counterclassical reactions to lung volume changes when light might have classical ones when deep. This evidently accounts in part for the shifts as previously described for figures 2 and 3. This is especially evident in figure 3. If the diminution in central chemical inspiratory drive that attends deepening anesthesia was entirely responsible for the changes in reaction to deflation and inflation in this experiment then there would have been no reversal of the deflation reflex response for the pulmonary vagal deflation drive is predominantly inspiratory and is therefore theoretically additive to the chemical drive. Therefore the slowing of breathing associated with a decreased lung

volume (neg. press.) that was accentuated by vagal block (A) must have been due to an extravagal expiratory reflex drive that was greater than the combined existing and building chemical inspiratory and the induced vagal inspiratory drives. Deepening the anesthesia evidently effected a much greater reduction of the extravagal drive than it did the vagal, inasmuch as the deflation response became classical at a moderately deep level, part B. It cannot be assumed that no depression of the pulmonary vagal drive takes place during deepening anesthesia as both the inflation and deflation drives are often definitely depressed as illustrated in figure 4. Compare B and C.

The ease with which these seemingly contradictory experiments can be reduced to a single simple thesis constitutes additional support of Gesell's concept of reflexogenic respiratory control.

#### SUMMARY AND CONCLUSIONS

Dogs *lightly* anesthetized with sodium evipal show great variability in their respiratory reactions to inflation and deflation of the lungs. When they are *deeply* anesthetized their reactions to pulmonary inflation and deflation are with but rare exception qualitatively similar: inflation slows and deflation speeds breathing. The change from variable to comparable responses is considered to be largely due to the more powerful depression effected by evipal upon the central chemical respiratory drive than upon the reflex drives; and to the greater depressant action of this agent upon the extravagal proprioceptive than upon the vagopulmonary reflexes.

The changes in character and efficacy of the vagopulmonary respiratory reflexes that attend variations in the depth of sodium evipal anesthesia make it impossible to define upon the basis of evidence obtained from animals anesthetized with sodium evipal the relative rôles that these reflexes play in the control and maintenance of normal breathing.

Of all the concepts regarding the functional characteristics of the Hering-Breuer reflexes that recently formulated by Gesell lends itself most readily and with fewer assumptions to an explanation of the changes that these reflexes undergo when the depth of sodium evipal anesthesia is varied.

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# CENTRAL STIMULATION OF RESPIRATION DURING HYPOXIA<sup>1,2</sup>

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Following the discovery of the peripheral vascular proprioceptive and chemoreceptive respiratory reflex mechanism (5), it appeared for a time that the chemoreceptor function of the respiratory center was to be shifted from the central nervous system to the specialized vascular structures. With but a few exceptions (6) (7) (8) (9) (27) it has been the finding that in the absence of the peripheral chemoreceptor mechanisms the anesthetized animal is incapable of making a satisfactory respiratory or circulatory adjustment to lowered partial pressures of oxygen in the inspired air (5) (21) (20) (2) (26) (28) (29) (15) (18) (4) (24) (10) (14).

Quite by chance, during the course of an investigation undertaken to attempt a comparative evaluation of the effects of various commonly used anesthetics upon the respiratory drive mechanisms, it was observed that a few animals under moderately light sodium evipal anesthesia, in which the cervical vagi had been severed and the carotid regions denervated, tolerated lowered alveolar oxygen tensions remarkably well; and in one experiment there appeared a fair hypoxic hyperpnea. This response was entirely abolished by a very slight increase in the depth of anesthesia. This led us to conduct six experiments (4 evipal; 1 pentothal; 1 cyclopropane) *for the purpose of determining whether or not the central hypoxic stimulation could be obtained with regularity* when special effort was expended to maintain the animal at the lightest level of anesthesia compatible with sound physiologic practice.

**METHODS.** Healthy, mongrel dogs weighing between 7.5 and 14 kgm. were anesthetized with sodium evipal (50 mgm. per kilo—10 per cent aqueous solution), with sodium pentothal (25 mgm. per kilo—5 per cent aqueous solution), administered intravenously, and with cyclopropane by inhalation. The general experimental methods have been described elsewhere (1).

The depth of anesthesia was objectively followed by recording the ipsilateral reflex contraction of the left semitendinosus muscle on stimulating the central end of the cut sciatic nerve, and also by noting the condition of the lid and corneal reflexes.

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The vagosympathetic trunks were cleanly cut with a very sharp, thin knife low in the cervical region. The carotids were denervated by double ligation and transection of Hering's bundle including the occipital artery. The common, internal, and external carotid arteries were stripped of their adventitia for a distance of at least two centimeters from the bifurcation. Absorbent cotton well saturated with one per cent procaine hydrochloride was then placed over the denuded bifurcation area.

**RESULTS.** The experiment previously mentioned is shown in figure 1, section 1. Ten per cent oxygen when given after vagal section during light anesthesia resulted in the hyperpnea shown in figure 1, section 1, A. Between A and B the carotids were denervated. No sodium evipal was given during the operation and consequently the anesthesia is lighter after carotid denervation. The rate of breathing had increased and respiration had lost the characteristics ordinarily seen after bilateral cervical vagotomy. The reaction to the same low oxygen mixture (10 per cent) differed from that previously obtained. Periodicity, a decreased tidal air, and an acceleration of rate which was capable of increasing pulmonary ventilation for a short time now appear. The gasping movements which were continuous during hypoxia when the carotid receptors were intact (A) were now intermittent and limited to the periods of greatest respiratory and vasomotor activity. They became continuous for the period of maximal amplitude after the hypoxia was terminated.

Experiment 38 (fig. 1, sec. 2) provides a more comprehensive picture of central hypoxic respiratory stimulation. The reaction of the fully innervated animal to hypoxia is shown in A.

Between A and B the cervical vagosympathetic trunks were severed and the carotids denervated. These procedures necessitated the administration of one cubic centimeter of sodium evipal. The administration of 8 per cent oxygen (B) provoked a fall in blood pressure and a diminution in breathing. Following the hypoxic period a definite increase in pulmonary ventilation occurred which exceeded the pulmonary exchange before the hypoxia. After a thirty minute wait the anesthesia had become almost as light as it was in A. Within the second minute of the low oxygen administration, C, respiratory stimulation began and rapidly increased. Shortly after the termination of the hypoxia a relative apnea occurred. However, one minute after the hypoxic period a respiratory minute volume of 26.4 liters was observed; this was greater than that seen at any time during the hypoxia and differed from the hypoxic hyperpnea in that amplitude played a greater rôle than formerly in its production.

Following this stimulation the carotid denervation was checked and found to be complete and more procaine was applied about the bifurcation. The vagi were also inspected and both found to be completely severed. The low oxygen administration was then repeated twice with the same result as shown in C. Eight-tenths of a cubic centimeter of sodium evipal was then given intravenously; breathing typical of vagotomy was established, and hypoxia evoked only a very transient stimulation and death.

Figure 2 shows the typical hypoxic reaction of the lightly anesthetized animal.

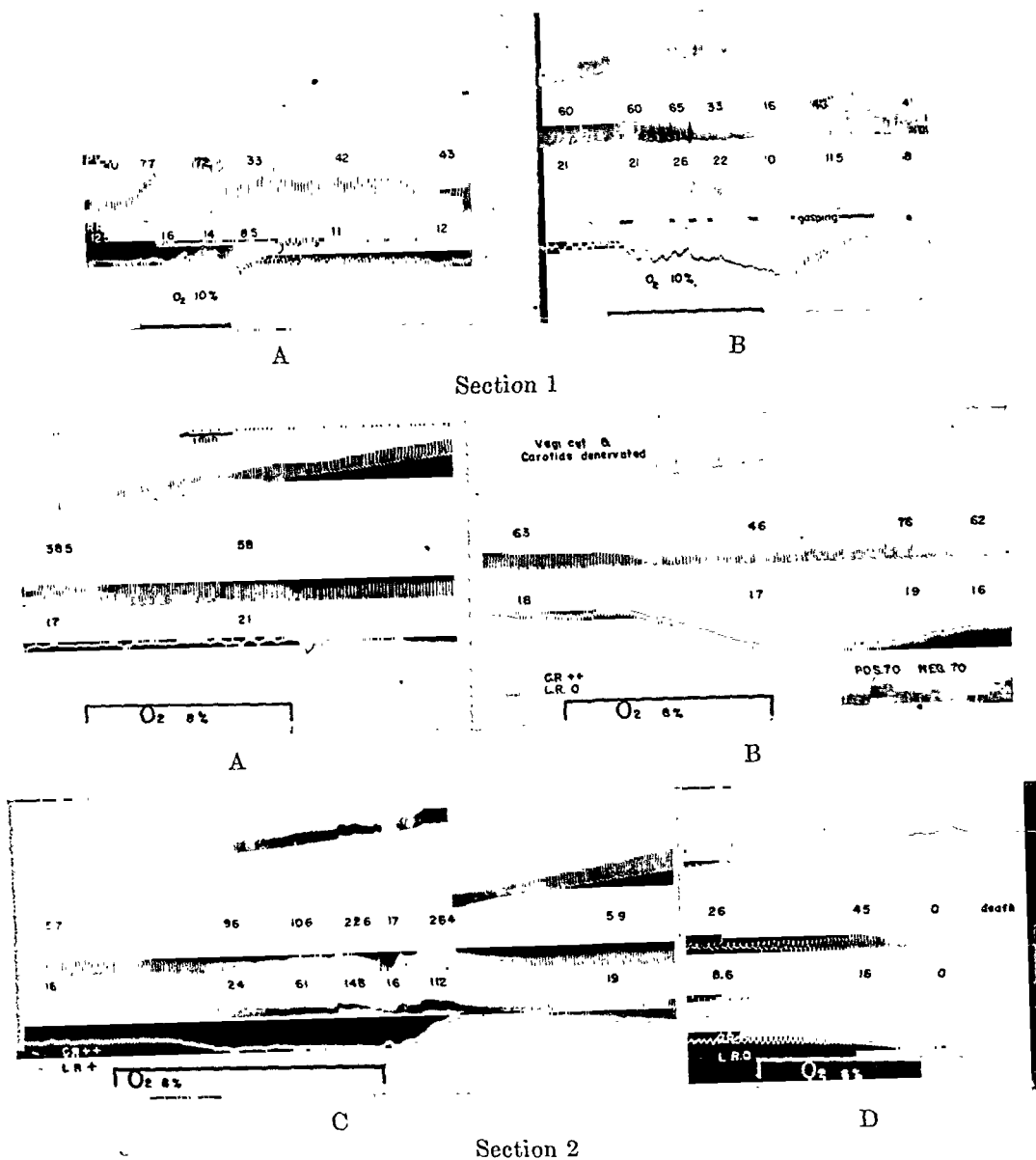
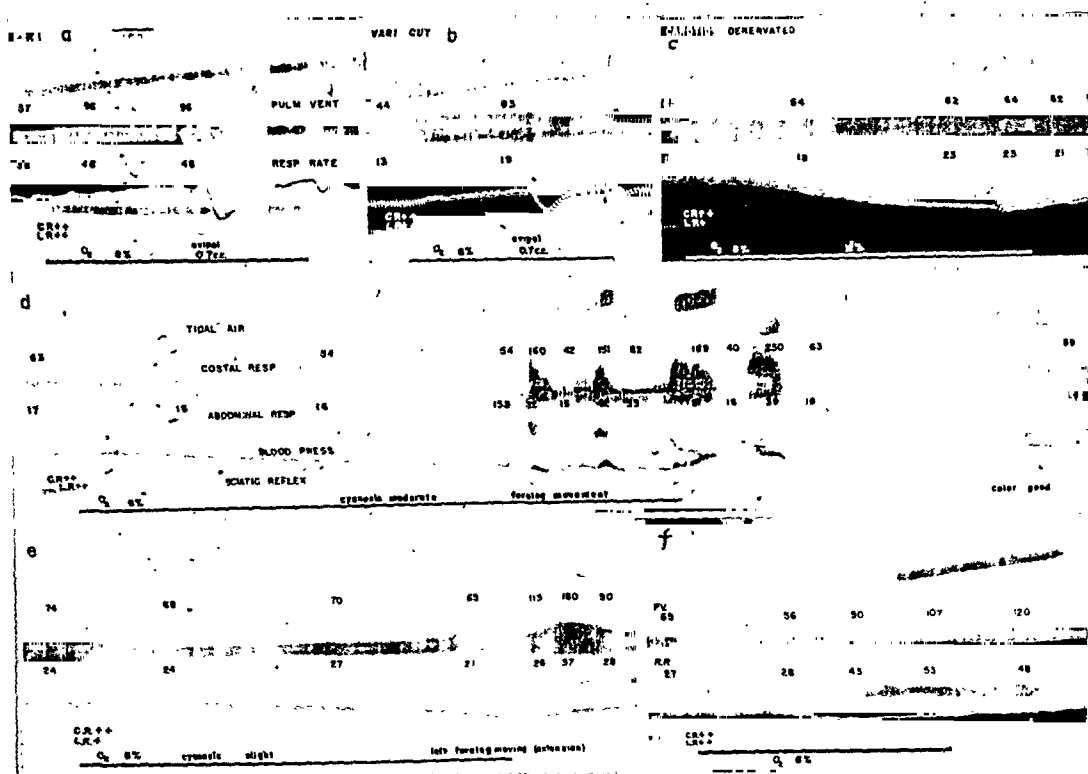


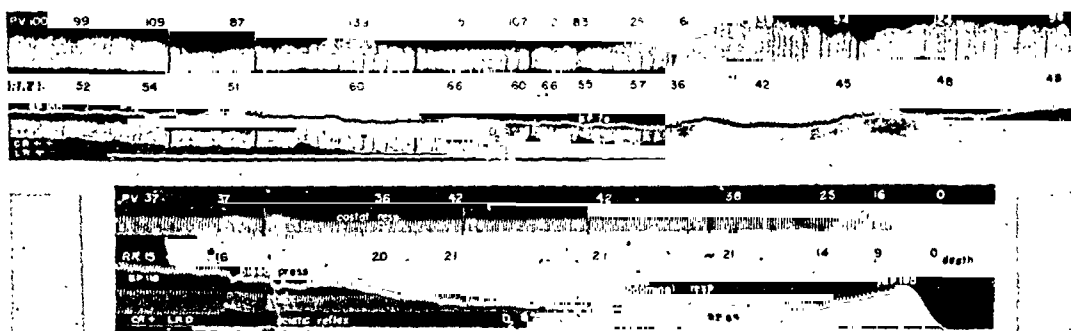
Fig. 1. The tracings from above downward indicate: Time in 5 sec. intervals with minutes shown; the tidal air respiratory excursion; *P.V.*, indicates pulmonary ventilation in terms of minute volume, expressed in deciliters; *C*, costal respiratory excursion; *R.R.*, respiratory rate; blood pressure; abdominal respiratory excursions; fine line shows sciatic reflex indicator, broad line indicates the interval during which special agents were administered; *C.R.*, corneal reflex; *L.R.*, lid reflex; signal marker. In the records of respiration an upstroke corresponds to inspiration; read from left to right. Except as specified the animal breathed room air. Section 1, vagi cut before A. Carotids denervated between A and B. Section 2, vagi cut and carotids denervated between A and B.

One and one half cubic centimeters of sodium evipal had to be given in order to cut the vagi and denervate the carotids; the anesthesia is, therefore, deeper in C than in A. The animal was then subjected to 8 per cent oxygen for nine minutes.

The response to hypoxia after denervation (C) was primarily one of depression. A short postanoxic hyperpnea was evident.



Section 1



Section 2. A, above; B, below

Fig. 2, Section 1. Details are as for figure 1, except denervations occurred as shown. Section 2, vagi cut and carotids denervated before tracings were made; A, light anesthesia; B, deep anesthesia.

The level of anesthesia was then allowed to lighten until it reached that present in A; low oxygen was again administered, D. During the eleventh minute of hypoxia a cyclic stimulation of breathing suddenly began. Coincident with

the periods of hyperpnea, blood pressure rose slightly, and running movements of the forelegs appeared; extreme jactitation was also present. Room air was given to the animal during the third period of hypoxic hyperpnea; all convulsive movements quickly ceased and respiration gradually slowed down. This was followed by a postanoxic hyperpnea that exceeded the hypoxic hyperpnea. It was unattended by general somatic muscular movement.

Following the injection of one cubic centimeter of sodium evipal, hypoxia was attended by the reactions shown in E. The oxygen deficiency was terminated after eleven and one-half minutes without a significant sign of stimulation. However, forty seconds after room air was given, definite signs of respiratory stimulation appeared. Pulmonary ventilation rose to a level which represented a 10.6 liter increase over normal.

The animal was then rested for twenty minutes after which low oxygen was given (F). After a two minute latent period a smooth stimulation of respiration attended the hypoxia; jactitation and somatic movements were *not* present. The rate increase was now a more prominent factor of the hyperpnea than at any time previously. The postanoxic hyperpnea is also evident and follows the hypoxic stimulation without sign of the preliminary depression. Two and one-half cubic centimeters of sodium evipal were then given and hypoxia then was attended by respiratory failure without any sign of stimulation (not illustrated).

One experiment was done under sodium pentothal anesthesia (fig. 2, sec. 2). All the records show hypoxic responses of the fully denervated animal. A demonstrates the rapidly augmenting character of inspiration after vagotomy under very light anesthesia; B shows the change to the slowly augmenting inspiration typical of vagotomy produced by only 0.7 cc. of 5 per cent sodium pentothal. The changes in the hypoxic reaction under sodium pentothal anesthesia as it is deepened appear to be in general the same as those described when sodium evipal is used as the anesthetic agent.

The one experiment performed under cyclopropane anesthesia had a result very like that under evipal and pentothal. The acutely denervated animal when anesthetized with 20 per cent cyclopropane showed great respiratory stimulation in response to oxygen deficiency; this was associated with generalized clonic convulsions. After deepening the anesthesia (cyclopropane, 30 per cent) oxygen deficiency evoked respiratory depression.

**DISCUSSION.** The hypoxic stimulation of breathing in the acutely denervated, lightly anesthetized animal differs from that of the normally innervated one under similar experimental conditions in that the latent period is prolonged and periodicity is more prominent. The postanoxic hyperpnea is a definite part of the picture in the denervated animal's hypoxic response, whereas it is only rarely seen in that of the normally innervated one.

During the latent period the respiratory changes of blood and tissues must be in a measure qualitatively comparable to those described by Gesell and associates (12) (13) as occurring in dogs subjected to low oxygen tensions while the animal's ventilation was kept uniform (controlled ventilation). Brassfield (3)



has recently shown that the  $C^{(H^+)}$  of the brain surface rises under like experimental conditions. It may be inferred from the experiments described here that biochemical changes of a similar nature very likely occur before the actual hypoxic hyperpnea begins, and that an increasing acidity of the cells of the center is probably the direct stimulus responsible for the increased respiratory activity coincident with the subjection of lightly anesthetized, acutely denervated dogs to decreased oxygen tension.

*In order to obtain any significant stimulation of respiration by hypoxia in the denervated animal it is necessary to have the animal very lightly anesthetized.* It is evident that only a slight increase in the depth of anesthesia will greatly reduce the ability of a denervated animal to make adjustments to the same degree of oxygen lack.

The level of anesthesia at which no, or only a slight, stimulation of breathing attends hypoxia in the denervated animal is in some cases too light to allow surgery to be performed. It therefore appears that the sensitivity of the center to  $(H^+)$  ions is very substantially lowered by a small amount of sodium evipal. The mechanism whereby evipal effects a very great reduction of the central stimulating action of  $(H^+)$  ions may be closely allied to, or identical with, its depressant action upon brain respiration in vitro. See Jowett and Quastel (16), and Quastel (19).

The postanoxic stimulation of respiration associated with the central hypoxic reaction seems to be pertinent to the question of the relative rôles played by  $C^{(H^+)}$  changes, and decreased oxidations (17) within the center in the chemically denervated animal. The postanoxic hyperpnea, which in all cases is greater than that present during hypoxia, likely is related to the accumulation of acid products of anaerobic glycolysis produced during the hypoxic period. The center may become more sensitive to these products when the hypoxic period is terminated, more sensitive, that is, as the oxygen tension within the tissues is increased. Should the increased oxidations produce carbon dioxide in excess of that necessary to balance the buffer base freed by oxidation and conversion of the products of anaerobic glycolysis, the acidity of the center might well increase. Be that as it may, it appears that the postanoxic hyperpnea occurs while central  $C^{(H^+)}$  may be decreasing (12). This change is generally considered as being adequate cause for a depression of respiratory activity. However, it appears likely that the increased oxygen made available to the respiratory neurones besides possibly effecting an increased carbon dioxide production serves to decrease the threshold of the center to  $(H^+)$  ions far more rapidly than the  $C^{(H^+)}$  is decreased. If this situation actually exists it would explain the presence of hyperpnea where relative apnea might be expected. Furthermore, decreased oxidations accompanying the hypoxia might be expected to prevent respiratory adjustments to an increased  $C^{(H^+)}$  should the sensitivity of the center be reduced by an anesthetic to a level which prevented the  $(H^+)$  ion stimulus from exceeding threshold strength at any time during the period of lowered oxygen tension. At this level of anesthesia, if severe damage has not been sustained by

the center during the period of oxygen lack, termination of the hypoxia should be attended by a stimulation of respiration. This actually occurred in two experiments, figure 1, sec. 2, B, and figure 2, sec. 1, E.

The long latent period of central hypoxic stimulation with its attendant depression of respiration also supports this thesis in that the respiratory depression occurs as the acid stimulus to breathing is increasing. It appears therefore that any increased respiratory activity that accompanies a given ( $H^+$ ) ion increase during hypoxia could be considered as indicating a maximal response to the  $C^{(H^+)}$  change by the center at a given level of oxidations and that the lower the oxidations, the smaller the respiratory response to a given increase in  $C^{(H^+)}$ .

The possibility that the stimulation of respiration attending hypoxia, in the absence of carotid and aortic and possible pulmonary chemoreceptor innervation, may be due to unknown chemoreceptive mechanisms cannot be ruled out by these experiments.

The occasional association of coördinated running movements, jactitation, and clonic convulsions (cyclopropane anesthesia) with the hyperpnea that attends hypoxia after complete chemoreceptor denervation suggests the excitation of motor centers besides the respiratory. These signs of generalized motor stimulation are seen under all states of innervation when the animals are very lightly anesthetized. When they do appear a carefully directed increase in the depth of anesthesia will entirely abolish them before the respiratory reactions are much reduced. In addition to this observation we have the fact that respiratory stimulation generally appears before the signs of general motor excitation (very occasionally they appear simultaneously) and considering further that some of the largest respiratory responses to hypoxia after denervation are entirely unattended by any sign of stimulation of other motor centers (fig. 1, sec. 2, C and fig. 2, sec. 1, F), it appears improbable that the stimulation of breathing is secondary to the stimulation of other motor centers. It seems reasonable to assume that the hyperpnea as well as the jactitation, running movements, and convulsions have a common stimulus, possibly an increasing cellular acidity.

However, it is not to be necessarily inferred from these experiments that the unanesthetized denervated animal will react in a similar manner, as there is a definite possibility, when the vagaries of all biological processes in anesthetic states are considered, that the central respiratory stimulation by oxygen lack may subsequently prove to be peculiar to the light anesthetic state.

An interesting observation is that the typical slow deep breathing following vagotomy is in most cases lost when the anesthesia becomes sufficiently light. This suggests the assumption of vagal function by respiratory muscle proprioceptive reflexes. One animal out of six, even when very lightly anesthetized, was unable to establish an eupneic type of respiration after vagotomy. All the others behaved as did the four described in this article. The observation that rapidly augmenting inspiration and a fast rate of respiration was the general finding in vagotomized animals when lightly anesthetized is in accord with the findings of Sharpey-Schafer (22) (23) and Tütso (25).

## SUMMARY AND CONCLUSIONS

Oxygen scarcity in the inspired air results in a significant sustained respiratory stimulation in lightly anesthetized animals<sup>4</sup> acutely deprived of known peripheral chemoreceptive and vagal proprioceptive reflex drive mechanisms.

The respiratory stimulation is generally preceded by a long latent period that is associated with depression of breathing and vasomotor pressor activity. The hyperpnea itself is characterised by periodicity, and a rapid rate. Readministration of room air is attended in most animals by a short period of respiratory depression. In all experiments, whether or not an immediate postanoxic depression occurs, a postanoxic hyperpnea obtains which differs from the hypoxic hyperpnea in that the attendant minute volume is greater. The postanoxic hyperpnea approximates in type the central stimulation of carbon dioxide far more than does the hypoxic response. The latter has the essential characteristics of anoxic stimulation in the innervated animal.

A very slight increase in the depth of anesthesia serves to reduce greatly or abolish the hypoxic response, but only diminishes the postanoxic hyperpnea. Further increase of anesthetic depth results in the often seen picture of hypoxia after complete chemoreceptive and vagal proprioceptive denervation, namely: progressive respiratory and circulatory depression that may be occasionally associated with a transient, inadequate increase in breathing.

The indirect evidence provided by these experiments appears to indicate that decreased oxidations within the center constitute a most important limiting factor of respiratory adjustments to changes in central  $C^{(H^+)}$ .

The occasional association of signs of generalized motor stimulation with those of respiratory center excitation is interpreted as indicating that functionally diversified motor neurones have a common sensitivity to increasing cellular acidity and not as indicating that the stimulation of breathing is secondary to increased activity of other motor centers.

Under sufficiently light sodium evipal and sodium pentothal anesthesia the rapidly augmenting inspiration of the normally innervated animal is present after vagotomy in the majority of animals. The slowly augmenting inspiration and slow rhythm described as being typical of breathing after vagotomy are always present when the anesthesia is sufficiently deep. It is suggested that respiratory muscle proprioceptives may take over the rôle played by the vagi if the anesthesia is light.

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# THE INFLUENCE OF THE PITUITARY AND ADRENAL CORTEX ON RESISTANCE TO LOW ENVIRONMENTAL TEMPERATURES<sup>1</sup>

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It is becoming increasingly apparent that adrenal function is intimately concerned with various physiological processes which enable the organism to withstand stress. Detailed information in this regard may be obtained in those forms of stress that can be experimentally controlled, such as exposure to low environmental temperatures. Many workers have observed that adrenalectomized animals are unusually sensitive to cold. For example, in 1914 Elliot (1) found that adrenalectomized cats would survive for longer periods if kept in heated cages. Belding and Wyman (2) noticed the greatest mortality among adrenalectomized rats during periods when there was a fall in temperature in the animal house. Cannon and his school (3, 4) had already pointed out the intimate relationship of the adrenals to temperature regulation, while Wyman and tum Suden (5) later found that cortical transplants in adrenalectomized rats restored to normal their ability to maintain a normal rectal temperature when exposed for two hours in a moderately cold room, while rats with cortical insufficiency were unable to maintain their normal body temperatures. This demonstrated the relatively greater importance in this regard of the cortex over the medulla. Hartman, Brownell and Crosby (6) observed that the administration of cortin enabled adrenalectomized rats to maintain their body temperature almost as well as normal rats when placed in a cold environment and Selye and Schenker (7) have recently made use of this phenomenon in the development of an assay for small quantities of cortin. This sensitive test enabled Weil and Browne (8) to detect cortin in human urine.

Hypophysectomized animals are similar to animals deprived of the adrenals in exhibiting an impaired capacity to maintain a normal body temperature when exposed to cold as first observed by Smith and Foster (9, 10). Baird, Cloney and Albright (11) found that cortin would protect hypophysectomized animals under those circumstances, and concluded that the adrenals were largely responsible for the maintenance of body temperature during cold exposure.

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<sup>2</sup> Aided in part by a grant from Roche-Organon Inc., Nutley, N. J.

Although it is well recognized that the hypophysis is essential to the maintenance of the normal size and histological structure of the adrenal cortex, little is known concerning the action of hypophyseal corticotrophin on the adrenal cortical function. As the normal resistance to a cold environment requires an adequate functioning of the adrenal cortex, it appeared likely that this reaction would serve as a measure of corticotrophic activity in the hypophysectomized animal. In the present study the atrophic adrenal cortices of hypophysectomized rats were stimulated by the injection of pituitary extracts and the influence of this treatment was then tested under the stress of exposure to low environmental temperatures.

**METHODS.** The experiments reported here were performed on hypophysectomized rats whose ages ranged from 35 to 40 days. The weight of the animals varied between 70 and 90 grams. Male animals were used in all cases except in individual experiments when the factor of sex was being investigated. The hypophysectomy was performed by a parapharyngeal approach according to Greep's modification (12) of the technique of Smith (9); injections were usually begun immediately after operation and continued twice daily until the end of the experiment; body weights were recorded daily. After various periods of treatment and from 0 to 51 days after the operation, the animals were placed in a refrigerator at  $0^{\circ} \pm 1^{\circ}\text{C}$ . and at a relative humidity of 70 to 75 per cent. Each animal occupied an individual compartment in a cage of  $\frac{1}{2}$  inch mesh wire which was designed to allow free convection of air and unobstructed radiation to all sides except toward the limited area occupied by the other animals in nearby compartments. No forced circulation of air was supplied.

In some experiments adrenalectomy or thyroidectomy was performed and in other instances more than one endocrine organ was ablated. A total of 643 animals was used, 57 unoperated animals, 459 hypophysectomized, 72 adrenalectomized, 33 hypophysectomized-adrenalectomized and 22 hypophysectomized-thyroidectomized. The exposure to  $0^{\circ}\text{C}$ . was interrupted at hourly intervals for the measurement of colonic temperatures. These readings were taken in a room at  $8^{\circ}\text{C}$ . and required a period of 5 to 10 minutes, depending upon the number of animals under test. The duration of exposure to low temperature varied from 1 to 8 hours and was usually discontinued prior to the death of the animals. When survival was desired the animals were removed to their accustomed room temperature of 26 to  $28^{\circ}\text{C}$ . before their body temperatures had fallen below  $15^{\circ}\text{C}$ ., otherwise subsequent fatalities were frequent. In individual experiments the animals were divided into two or more equal groups, so that untreated animals served as controls for each determination. This procedure was essential because tests upon similarly prepared animals on different occasions yielded dissimilar temperature curves no matter how carefully the above precautions were followed. This variation is shown in the figures below, but the source of the phenomenon has not been uncovered. One variable which could have been controlled more rigidly was the length of time required to read body temperatures. On the whole a longer period was required in the  $8^{\circ}\text{C}$ . room when larger groups were used and consequently these groups were less rigorously chilled than smaller groups.

At autopsy the adrenals, the thyroids, the gonads and the ventral prostates were weighed and the completeness of the hypophysectomies was checked by an examination of the sella turcica under a dissecting microscope. At the same time the base of the brain and the diaphragm of the sella turcica were carefully examined for signs of damage. Excluded from the data were animals incompletely hypophysectomized or animals in which the diaphragm had been ruptured during operation resulting in damage to the base of the brain. Such animals usually showed an inability to maintain their body temperature regardless of whether they had been treated or not.

In experiments where the significance of observed differences were questionable, Fisher's formula was applied and in the following discussion only those differences yielding a P value of 0.05 or less are stated to be significant.

Corticotrophic extracts were prepared from the whole pituitary glands of sheep. Freshly frozen tissue or commercial acetone-dried powders served as starting material. For the sake of satisfactory yields of the active material, acid or alkaline extraction media were employed at pH 1.5 to 3 or 8.0 to 10.0, respectively. The degree of purity and the freedom from other pituitary hormones varied widely in different preparations and no preparation used could be considered to be entirely pure. The preparation used in many of the experiments was made from fresh glands by the repeated extraction at pH 2 in 60 per cent acetone and subsequent precipitation of the active material with 4 volumes of acetone. This preparation was not further purified and is referred to below as crude acid acetone extract. Other extracts were prepared by the method of Lyons (13) but differing to the extent that no iso-electric precipitation of corticotrophin at pH 6.5 was performed. These extracts contained luteotrophin but were free of F.S.H., L.H. and thyrotropin and did not promote growth. Later experiments were made with more highly purified preparations virtually free of luteotrophin. In each case the dosages are expressed as weight equivalents of original acetone-dried whole pituitary powder. In this calculation it was assumed that the weight of the commercial dry powder was 20 per cent of the wet weight of the fresh gland. The corticotrophic activity of these extracts was determined by the adrenal weight increase in hypophysectomized animals injected twice daily for 5 days. In some cases these assay animals were tested in the cold immediately prior to autopsy.

**EXPERIMENTAL.** A number of groups of untreated male rats were exposed to cold under the above conditions at various intervals after hypophysectomy. The rates of fall in body temperature are plotted in figure 1, each curve representing the average temperature change of a group of 4 to 19 animals. In no case were these animals exposed to the cold more than once. It is apparent that determinations made on different occasions vary considerably even though like conditions as to age, size of the animals, temperature of the environment, and so forth, prevailed. The increased sensitivity to cold on the 4th and 28th day after operation is manifest, however, as is the striking resistance 24 hours after the operation. Animals exposed immediately after hypophysectomy were somewhat inferior to the 24 hour groups in their capacity to withstand the

exposure and were similar in their behavior to the groups tested 48 hours post-operatively. One group of adrenalectomized animals exposed on the day after operation is included for comparison. It would appear from these data that four days are required for the hypophysectomized animals to develop a degree of sensitivity to cold comparable to that of animals deprived of their adrenals. Female rats tested under these same conditions were found consistently to be more resistant to cold than males.

The influence of adrenal cortical extracts and of desoxycorticosterone acetate was tested on hypophysectomized and on adrenalectomized animals in the following manner. On the evening prior to the test and shortly after operation one injection of 1 cc. of cortin<sup>3</sup> or 0.5 mgm. of desoxycorticosterone acetate<sup>3</sup> in

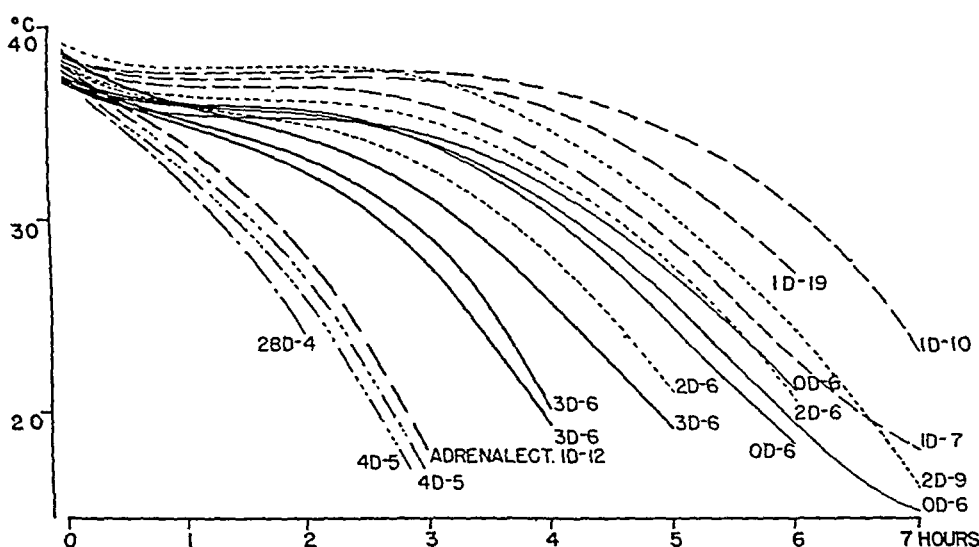


Fig. 1. Average rate of fall of the body temperatures of untreated hypophysectomized male rats exposed to an environmental temperature of  $0^{\circ}\text{C}$ . The numbers of animals used in each group is given.  $0D$  = on the day of hypophysectomy,  $1D$  = 1 day after hypophysectomy, and so forth. One group of adrenalectomized animals, exposed to cold one day after operation, is shown for comparison. The animals were most resistant to cold on the day following operation and reached their maximal sensitivity three days later. All animals were exposed to cold on one occasion only.

0.1 cc. of oil was given. A second like injection was made immediately before the animals were exposed to  $0^{\circ}\text{C}$ . Figure 2 shows the rate of temperature fall in 6 groups of animals. The cortin and desoxycorticosterone acetate treatment significantly increased the resistance of adrenalectomized animals. In the hypophysectomized animals cortin was definitely effective in this regard but in this experiment at least desoxycorticosterone was without effect.

In an extensive series of experiments hypophysectomized rats have been treated with pituitary extracts for periods of 1 hour to 14 days and their behavior in the cold environment compared with that of control untreated ani-

<sup>3</sup> The cortin and desoxycorticosterone acetate (doca) used in the experiments were kindly supplied by Roche-Organon, Inc., Nutley, N. J.



imals. The effect of the crude acid acetone extract in four experiments is shown in figure 3. A single injection of 25 mgm. equivalents of pituitary powder immediately after operation and 1 hour before exposure proved effective in exerting a statistically significant protection as shown in figure 3A. A similar response was noted in the animals shown in B which received two injections during the 24 hours between operation and exposure. A greater difference between control and injected animals was noted when treatment was continued for 2 and 3 days, respectively, in experiments C and D. It is of interest to note that the average adrenal weights shown in A and B of the treated and control animals are identical and that no significant increase in adrenal weight occurred until after 3 days of treatment at this dosage level.

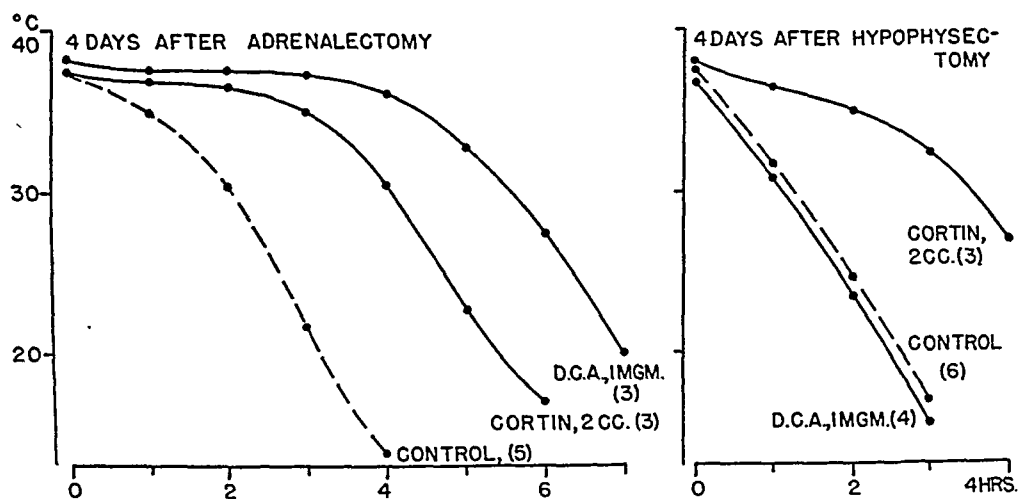


Fig. 2. The effect of cortin and desoxycorticosterone acetate on the rate of fall in body temperature of adrenalectomized and of hypophysectomized rats exposed to  $0^{\circ}\text{C}$ . During the 4 days between operation and exposure to cold the adrenalectomized animals were given 1 per cent NaCl in their drinking water. Two injections were made, 12 hours and immediately before the exposure to cold. The total doses are given in the figure. Protection was afforded both types of animals by cortin, but only the adrenalectomized animals were affected by desoxycorticosterone acetate.

When it was noted that considerable variation occurred between similarly prepared groups of animals it seemed advisable to introduce a further control measure. Prior to treatment hypophysectomized animals were exposed to cold and then on the basis of their performance in this preliminary test they were divided into two groups. One group was treated and the other saved as control. In 5 of 6 such experiments the less resistant group was injected with corticotrophic extract while the more resistant animals were allowed to go untreated. In this way a comparison could be made between injected and control groups as well as between the performances of the individual animals in the two tests. The data obtained in this way are given in figure 4. In experiments A, B and C the untreated animals were more sensitive to the cold on the second exposure while in D, E and F the untreated animals had already reached their maximal sensitivity when the first test was made 6, 21 and 28 days after operation. The

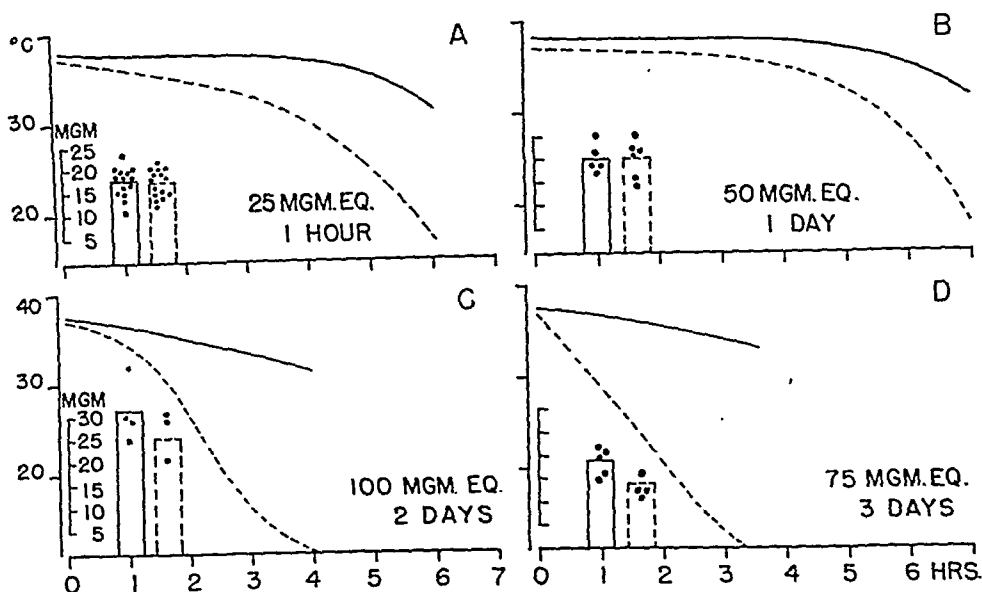


Fig. 3. Influence of crude pituitary extracts on the loss of body temperature of hypophysectomized rats exposed to 0°C. The treated animals (solid lines) were better able to maintain their temperature even when given a single injection one hour prior to exposure, but no adrenal weight change occurred until after 3 days of treatment. Young male animals of 70 to 90 grams were used in A, B and D; adult males of 270 grams in experiment C.

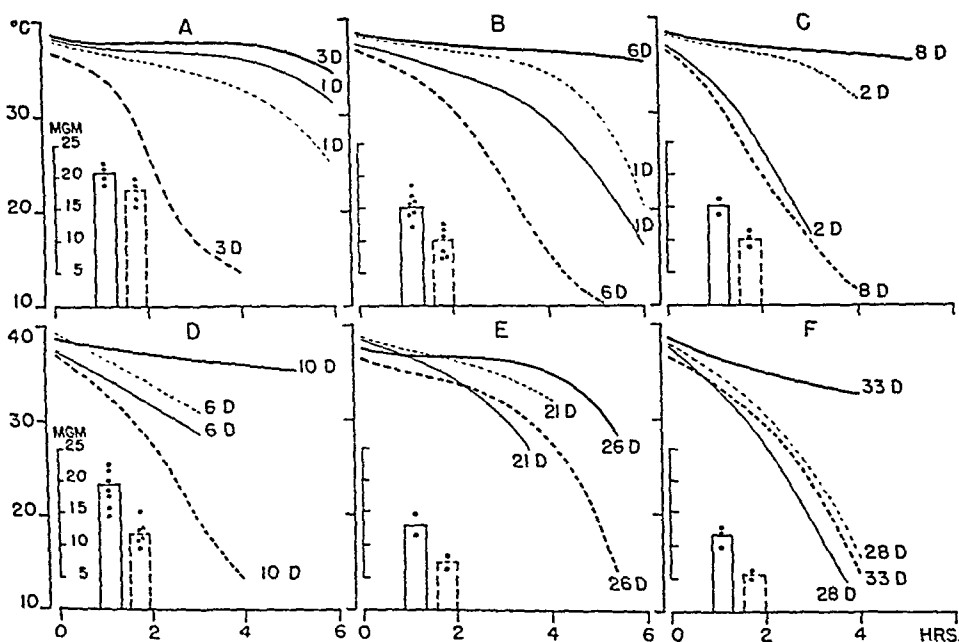


Fig. 4. Temperature curves of 6 groups of rats exposed to cold on two occasions. Between the first and the second exposure one half of the animals in each group were treated twice daily with corticotrophin. The performance of the treated animals is shown in heavy solid lines and of the untreated in heavy dashed lines. The behavior of these same animals on the preliminary test is shown in light solid and dashed lines, respectively. The number of days that elapsed after hypophysectomy is given in each case. The rectangles represent the mean adrenal weight in each group. Males were used in experiments A, B, F, and E, and females in C, D and E.

injected animals showed improved temperature maintenance after treatment in each instance, irrespective of their performance in the preliminary test.

The experiments recorded above were performed with either the crude acid-acetone extracts or the extracts prepared by Lyons' method. Other confirmatory tests have subsequently been made using more purified preparations known to be free of F.S.H., L.H., thyrotropin, luteotrophin and the growth principle. However, assays have not been performed to exclude the presence in these extracts of the several postulated metabolic principles. Consequently further experiments were considered desirable in order to establish the site of action of the factor or factors concerned in this increased tolerance to cold. The first

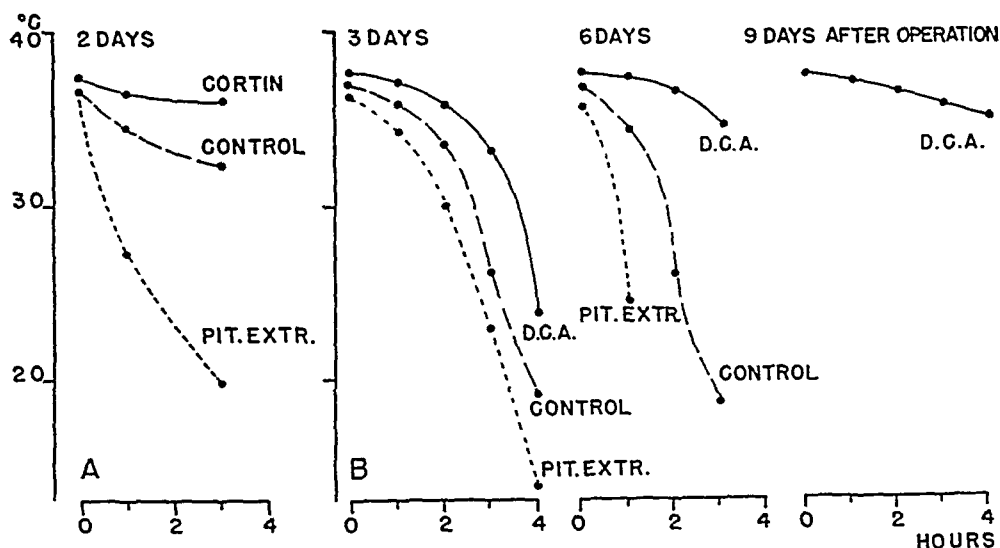


Fig. 5. A. Influence of a crude pituitary extract and cortin on the loss of body temperature of adrenalectomized rats, exposed to an environmental temperature of 8°C. two days after operation.

B. Effect of a crude pituitary extract and of desoxycorticosterone acetate on other groups of adrenalectomized rats exposed 3 times to 8°C., on the 3rd, 6th, and 9th successive day after operation.

Each group represents the average of 4 animals. Injections of 0.5 cc. of cortin, 0.25 mgm. of desoxycorticosterone acetate and of 25 mgm. equivalents of sheep pituitary powder, respectively, were made twice daily.

requirement was to determine the influence of pituitary preparations on adrenalectomized animals. Two such experiments performed at 8°C. are shown in figure 5. In A are shown three groups of animals. Cortin treatment in one group increased their resistance over the controls while the crude acid-acetone extract of proven efficacy in hypophysectomized animals, was deleterious. In figure 5B are shown three groups of animals exposed on three occasions. A similar response to that shown in A was observed after three days of treatment with desoxycorticosterone acetate. The difference became more marked after a further period of 3 days, and by the 9th day only the desoxycorticosterone-treated group still survived. Similar results were observed in repeated experiments with exposure of the animals to 0°C.

Although these data were strongly suggestive of an adrenal mediation of the observed action of pituitary extracts in hypophysectomized animals, they did not exclude the possibility that a part of the protection afforded hypophysectomized animals was centered elsewhere. It appeared highly probable that if the injected pituitary extracts exerted a protective action against cold in hypophysectomized animals by some other route than via the adrenal cortex such an action would be detectable in animals exposed to cold after both the adrenals and hypophysis had been removed. The data of figure 6, however, fail to show that any protection is afforded such doubly operated animals. Animals prepared in this way were highly sensitive to cold; they were protected by either cortin and desoxycorticosterone acetate but not by a crude pituitary extract or by a purified preparation of corticotrophin.

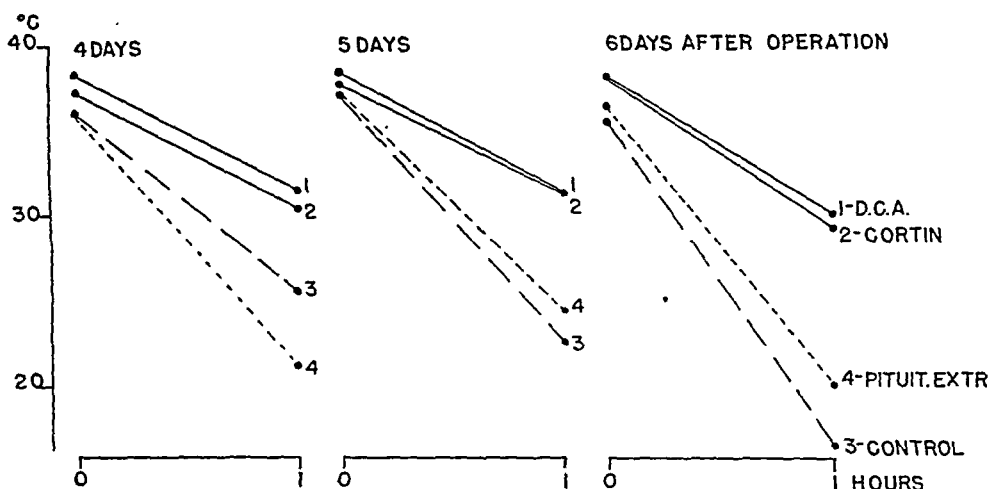


Fig. 6. Temperature curves of hypophysectomized-adrenalectomized rats injected with desoxycorticosterone acetate, cortin, and a crude pituitary extract, respectively, and exposed 3 times to  $0^{\circ}\text{C}$ ., on the 4, 5 and 6th successive day after operation. Each group represents the average of 4 animals. Injections of 0.5 cc. of cortin, 0.25 mgm. of desoxycorticosterone acetate and of 25 mgm. equivalents of sheep pituitary powder, respectively, were made twice daily for the 6 days of the experiment. All animals were allowed a 1 per cent NaCl solution between tests.

A similar series of experiments was performed on animals thyroidectomized at the time of hypophysectomy. These two operations likewise resulted in a very low resistance to cold. However, in the absence of the thyroid the pituitary extracts were effective in increasing both the weight of the adrenals and the cold tolerance of the animals. Treatment of 9 animals twice daily for 6 to 10 days with 25 mgm. equivalent of acetone-dried sheep pituitary powder resulted in a 50 to 87.5 per cent increase in the adrenal weights over those of 10 controls. After exposure to  $0^{\circ}\text{C}$ . for two hours the average body temperature of the three injected groups was  $30.7$ ,  $30$  and  $29.8^{\circ}\text{C}$ ., and that of the corresponding control groups  $23$ ,  $23.9$  and  $19.5^{\circ}\text{C}$ ., respectively.

**DISCUSSION.** The importance of a great many factors in temperature regulation is attested by the experience of numerous investigators. The present ex-

periments deal with the importance of the anterior pituitary and the adrenal cortex to the exclusion of other influences. Incidental to the experiments recorded above observations were made on certain other factors of importance. For example, animals younger than those used above exhibited a poikilothermic tendency which made them unsuitable for the tests. Large animals were better able to withstand cold than small animals, as were animals with a heavy growth of fur. Females were consistently better able to withstand cold than males. Lesions of the central nervous system accidentally inflicted during removal of the pituitary induced a sensitivity to cold which was more striking than that produced by any other single manipulation. These factors had to be excluded in order to obtain consistent results.

The experiments reported above indicate that the injection of pituitary extracts increases the resistance of hypophysectomized rats to cold. Purified extracts high in corticotrophic activity had an effect similar to cruder preparations and in either case no effect was observed in the absence of the adrenals. This is evidence for the belief that pituitary extracts exert their protective action through the adrenal cortex and that the enhanced adreno-cortical function is responsible for the increased tolerance to cold.

It is to be emphasized, however, that this treatment did not restore a normal response to low temperatures. The untreated hypophysectomized animals were unable to maintain their body temperatures above a lethal level for longer than 3 to 8 hours. Pituitary extracts permitted survival for perhaps twice this period but the capacity of treated hypophysectomized animals to withstand an environment of 0°C. falls far short of the 24 to 48 hours that can be tolerated by an unoperated untreated rat. Apparently, therefore, the removal of the hypophysis imposes a sensitivity to cold that is not completely corrected by the injection of pituitary extracts, at least not by extracts of the type and in the quantities used here.

The quite reasonable assumption that the pituitary may exert a protective influence against cold by some other route than over the adrenal cortex did not receive support from the experiments on adrenalectomized animals or on animals lacking both the hypophysis and the adrenals. Under such conditions pituitary extracts were without effect in increasing cold tolerance.

Many investigators have minimized the importance of the thyroid in increasing resistance against cold. The above data support this, for although thyroidectomy further increased the sensitivity of hypophysectomized animals, the absence of both glands did not prevent the protective action of pituitary extracts.

The experiments here reported confirm that immediately after hypophysectomy an increased sensitivity to cold is manifest. However, during the first 3 days postoperatively a certain resistance is retained and only after 4 days is the permanent high sensitivity to cold attained. This slow development of cold intolerance may be related to the similarly slow atrophy of the adrenal cortex described by Smith (9), and may indicate that some degree of adrenal cortical function continues even though anatomical regression is in progress.

It is of interest that a protective action against cold was noted immediately

after a single injection of corticotrophin. Such a result lends support to the current belief that in the normal animal the adrenal cortex responds promptly to stress by an increased activity and that this effect is brought about by an increased secretion of corticotrophin by the pituitary.

On the other hand the adrenal cortex retains its responsiveness to corticotrophin for many weeks after hypophysectomy as shown by the experiments in which both function and size was restored by pituitary extracts as long as 28 days after operation.

It has been shown (Ingle, 14; Selye, 15) that the hypertrophy of the adrenal cortex in the normal animal under conditions of stress may be considerable, amounting to a 25 per cent weight increase in the first 12 hours. The amount of corticotrophin given hypophysectomized animals in the present experiments was insufficient to bring about a comparable degree of hypertrophy, and it is reasonable to believe that herein lies the cause of failure to induce a normal resistance to low environmental temperatures.

#### SUMMARY

Hypophysectomized young rats weighing 70 to 90 grams were unable to maintain their body temperature when exposed to an environment of 0°C. Such animals became progressively more sensitive to cold over the first 4 post-operative days and thereafter exhibited a fairly constant response to cold. Crude pituitary extracts or purified corticotrophin increased the cold resistance of such animals, when administered for periods of from 1 hour to 14 days prior to exposure. These extracts were ineffective in the absence of the adrenals but were active after thyroidectomy. Adrenal cortical extracts increased the resistance of hypophysectomized rats and of similar animals without thyroids or adrenals. The protection against cold afforded hypophysectomized rats by pituitary extracts paralleled their corticotrophic activity and was attributable to the cortical function thereby induced.

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# EXPERIMENTAL INVESTIGATION ON THE EFFECTS OF TRAUMA AND TRAUMATIC SHOCK ON GASTRO-INTESTINAL MOTILITY AND SECRETIONS<sup>1</sup>

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Little is known about gastro-intestinal motility and secretions during traumatic shock. Patients in this condition are known to vomit frequently, but this effect does not necessarily imply activity of the stomach. Moon has said that atony of the gastro-intestinal tract prevails during traumatic shock (1). In a recent study on the effects of burns we have found a great increase of gastric motility and, in a number of animals, a considerable increase in the volume and acidity of gastric secretion (2). We were, therefore, interested in investigating the effects of noxious agents other than burns, and report in this paper our findings of the results of mechanical trauma on gastro-intestinal functions.

**EXPERIMENTAL PROCEDURES.** Normal, healthy dogs were fasted for 30 hours, but had access to water. They were anesthetized with pentobarbital sodium or with ether and, following a suitable control period, trauma was administered to one or both hind legs by blows. Unless the animals died during the course of the experiment they were destroyed at its termination with an overdose of anesthetic. Blood pressure was recorded from the carotid artery with a mercury manometer, respiration by a cuff around the chest; intra-abdominal pressure by a balloon placed between liver and diaphragm, and gastro-intestinal motility by balloons placed in various organs. Manometers with a mixture of oil and carbon tetrachloride with a specific gravity of 1.5 were employed. For the collection of gastric secretion esophagus and pylorus were ligated, the latter just beyond the sphincter, and the contents of the stomach measured and titrated at the termination of the experiment. For the collection of other secretions the following ducts were cannulated: submaxillary duct, common duct with the cystic duct ligated, and main pancreatic duct. The secretions were recorded by an instrument described by us (3).

**RESULTS.** A. *Secretions.* a. *Salivary, Pancreatic and Biliary Secretions.* *Controls.* In 4 control experiments without trauma intravenous infusion of glucose (5 per cent)-saline (0.9 per cent) at the rate of 2 cc. per minute was administered by a constant injection pump previously described (4). Hourly intravenous injections of pilocarpine nitrate 0.25 to 0.5 mgm., or of secretin,<sup>2</sup> 5 to 10 mgm., were given alternately, and in the following "change of secretion"

<sup>1</sup> Aided by the Rosetta Josephson Fund.

Read as preliminary report at the 1941 meeting of the American Physiological Society.

<sup>2</sup> We are obliged to Dr. D. Klein, Wilson Laboratories, for a supply of secretin.

denotes changes in stimulated secretions. Salivary secretion did not change (3 expts. 12, 12 and 6 hrs.). Bile secretion did not change in one experiment (12 hrs.) and a slight decrease occurred in two (12 and 4 hrs.). Pancreatic secretion did not change in 3 experiments (12, 12 and 4 hrs.).

In all animals, controls and traumatized, blood pressure fell gradually as the experiments progressed, but the animals maintained a mean blood pressure of about 100 mm. Hg during the greater part of the experiment.

*Trauma (19 expts.).* Constant intravenous infusion was administered, as described above. In experiment 1, no drugs other than the anesthetic were used. Following trauma, biliary secretion dropped from 20 to 6 drops per 15 minutes,<sup>3</sup> pancreatic secretion from 1 to 0, salivary secretion from 1 to 0, and blood pressure from 140 to 90 mm. mercury. At the end of the experiment 0.5 mgm. of pilocarpine was injected intravenously and salivary secretion rose

TABLE 1  
*Gastric secretions following trauma*

EXPERIMENT NUMBER	CONSTANT INFUSION PER MINUTE	BLOOD PRESSURE		GASTRIC SECRETION					REMARKS
		Before trauma	After trauma	Hours	Volume		Acidity		
					Total	Per Hour	Free	Total	
	cc.	mm. Hg	mm. Hg		cc.	cc.			
1	6			4½	15	3.3	0		Starved
2	6			4½	20	4.4	8	21	Starved
3	7			5½	18	4	0		Starved
4	6	165/105	80/40 to 135/100	5	20	4	18	33	Starved
5	5	160	135 to 100†	5	Fecal material in stomach				Starved
6	7	165	75 to 125†	5	25	5	0	10	Fed
7*	6	115	40 to 100†	5	10	2	0	10	Fed
8	7	185/115	105/80 to 160/110	5	25	6	14	41	Fed
Averages.....				4.9	16.7	3.4			

\* No. 7, ether; all others, nembutal anesthesia.

† Mean pressures.

to 54 drops, indicating that the potential function of the submaxillary gland had not been lost. A similar experiment was carried out on dog 2. Biliary secretion dropped from 15 to 9, pancreatic secretion from 1-3 to 0, salivary secretion from 20 to 0, and blood pressure from 120 to 40. In experiments 3-5, pilocarpine and secretin were administered as described under "controls." In experiments 3 and 4 pancreatic and salivary secretions were affected slightly or not at all by traumatic shock, while biliary secretion showed a 50 per cent decrease. In experiment 5, pancreatic secretion was not changed, while biliary secretion dropped 50 per cent.

b. *Gastric Secretion.* (Table 1.) Control experiments without trauma were performed on ten animals, in order to test the effects of anesthesia and operation. The rate of gastric secretion varied between 2.1 and 3.4 cc. per hour, with an average of 3.0 cc. No free acid was present in 9 experiments, and a trace of

<sup>3</sup> In the following the figures for secretion represent 15 minute periods.



free acid in one. Total acidity varied between 10 and 20 degrees (one degree represents cubic centimeters of  $n/10$  HCl per 100 cc. of gastric juice). The constant intravenous infusion of saline-glucose solution or feeding 10 hours before the experiment did not affect volume or acidity of gastric secretion, the average rate of secretion being 3.4 cc. in 7 animals.

Eight dogs under nembutal or ether anesthesia received constant intravenous infusion of saline (0.9 per cent) glucose (5 per cent). Four to five hours following the trauma the stomach was excised and its contents collected. The hourly rate of secretion varied between 2 and 6 cc. A small amount of free acid, varying between 8 and 18 degrees was found in 3 experiments; total acidity varied between 10 and 41 degrees. Previous starvation for 30 hours, or feeding 12 hours before the experiment, did not seem to affect acid secretion following trauma. A comparison with controls without trauma shows that the volume of secretion was not affected by trauma, the average hourly rate of secretion being 3.4 cc. in both series. We do not feel that the small amount of free acid found in 3 of the experiments with trauma was significant.

c. *Motility*. (Figs. 1 and 2.) Five dogs under nembutal and one under ether anesthesia were used. Blood pressure was recorded from the carotid artery, and balloon records of the motility of the distal part of the stomach (antrum) and, in some dogs, also of the body of the stomach, of the duodenum and of the colon were obtained. In one animal the motility of the exposed stomach was observed directly. After a suitable control period trauma was administered to the legs.

In 3 experiments (nembutal) antrum motility and tone were increased for short periods following trauma; in one of these experiments, in which colonic and duodenal motility was registered also, an increase of these motilities was noted. In 2 experiments (one nembutal, one ether), antral and duodenal tone and motility were diminished following trauma. In one of these experiments a slight and short increase of antral motility appeared after the sixth and seventh trauma. Following another traumatization, depression of antral motility occurred.

Figures 1 and 2 represent two typical experiments. In the experiment of figure 1, antral motility became greater after additional traumatizations, and when blood pressure was rather low, i.e., at 50 mm. Hg and below; at this stage, manipulation of the traumatized limbs was followed by prolonged antral motility.

In experiments in which antral motility was observed to follow trauma, the degree and duration of this motility was rather small as long as blood pressures were above 80 mm. As the experiment progressed, and further trauma or manipulation of the traumatized areas reduced blood pressures to 50 and below, motility of the antrum became stronger and more prolonged. The effects of trauma on antral motility were inconstant, however. This is demonstrated in figure 2.

In the experiment in which the stomach was observed directly, a slight increase of tone and motility appeared 5 minutes after traumatization. Most

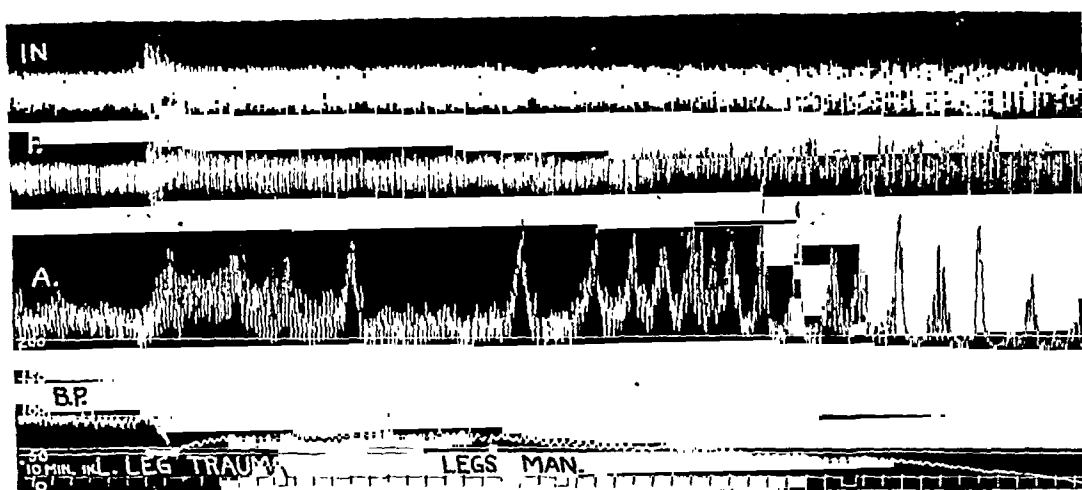
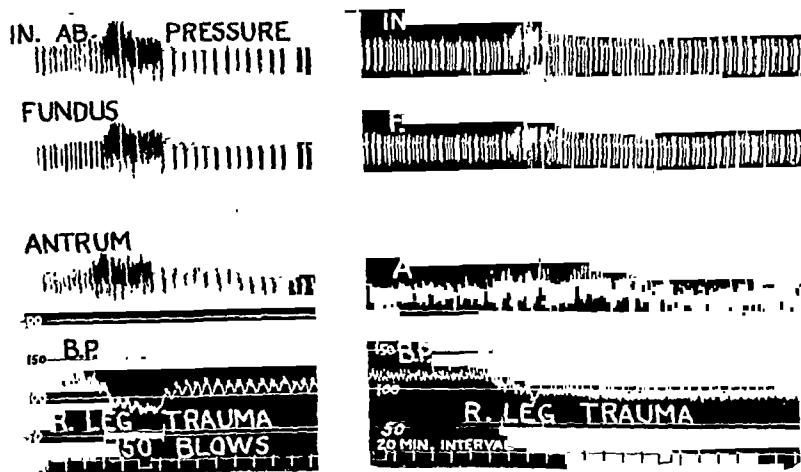


Fig. 1. Effect of trauma on gastric motility. Distinct increase of antral tone and motility following trauma to left leg and manipulation of traumatized legs; note low blood pressure. Nembutal anesthesia.

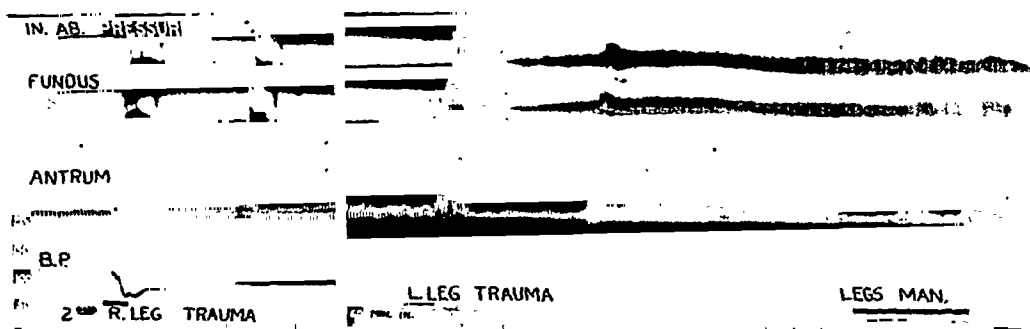


Fig. 2. Effect of trauma on gastric motility. Distinct depression of motility of the antrum following trauma of right and left leg. Ether anesthesia.

waves of contraction were seen in the body of the stomach, and fewer in the antrum.

Intra-abdominal pressure and respiration were increased slightly and for short periods of time following the trauma in some animals, while in others it was depressed.

Following trauma, slight edema and some petechial hemorrhages were found in the mucosa of the stomach and duodenum of two animals. A varying degree of anemia of the stomach and intestines was found, apparently depending on the severity of trauma and shock. The adrenals did not show gross changes.

DISCUSSION. In the experiments in which secretion had not been stimulated, salivary, biliary and pancreatic secretions decreased, while in the experiments in which secretion had been stimulated, salivary and pancreatic flow were not changed, but biliary secretion diminished 50 per cent. Secretin was not a particularly strong stimulant for biliary secretion, however.

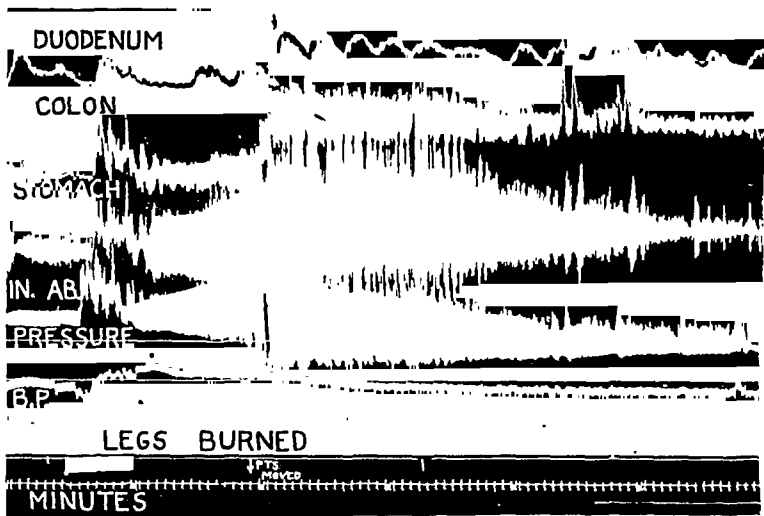


Fig. 3. Intra-abdominal pressure following burn. Nembutal anesthesia

We feel that changes in stimulated secretions may give more information about the function of an organ, because the periodic activities of most glands, as well as the effects of local changes in blood flow, may obscure functional changes. The depressive effect of burns on salivary, pancreatic and biliary secretions was considerably greater than that of mechanical trauma (2).

Gastric secretion was not affected significantly, neither in volume, nor in acidity, by various degrees of traumatization and ensuing shock. Gastric motility was slightly increased for short periods of time when the blood pressure of the traumatized animal was above 80 mm., and for somewhat longer periods and to a greater extent, when the blood pressure declined further. The intensity of gastric and mainly of antrum motility following trauma did not compare with that following burns. In the latter case it was seen mainly in the antrum, it was very forceful, began immediately with the burning and usually continued for prolonged periods of time. In no burn experiment was depression

of gastric motility observed, as seen in a number of experiments with trauma, but strong gastric motility was seen to follow every burn.

There is a considerable difference also between respiration following trauma or burns: with trauma, respirations may be inhibited or augmented, and in the latter case, the increase is of short duration and never as marked as with burns. Following burns, respiration is forceful and of great depth, and intra-abdominal pressure is greatly augmented (2); the latter never was seen to follow trauma in any of the experiments reported in this paper, nor in 50 similar experiments not reported here. The increased tone and contractions of the thoracic and abdominal musculature which were observed in nearly all burn experiments, never occurred with trauma. For comparison, the considerable increase of intra-abdominal pressure and respirations following a burn is demonstrated in figure 3 (unpublished experiment. Nembutal anesthesia).

There are thus fundamental differences between the manifestations of shock following trauma and burns, i.e., in respiration, intra-abdominal pressure, gastro-intestinal motility and gastric, pancreatic and salivary secretion. This demonstrates that although changes in circulation and composition of the blood may be similar in every case of fully developed shock, whatever its cause, other manifestations may be different. We believe that gastric motility following traumatic shock may be related to anoxemia, as it became more apparent with lower blood pressures. Of course, anoxemia of the stomach may be more dependent on vasomotor regulation than on systemic blood pressure, but experimentation only will be able to answer this.

#### SUMMARY

The effects of mechanical trauma on gastro-intestinal functions were studied in anesthetized dogs: stimulated salivary and pancreatic secretions were not affected much, but biliary secretion decreased. Trauma did not affect gastric secretion, and its effects on gastric motility were slight and variable. The effects on respiration and intra-abdominal pressure were variable and of short duration.

The effects of mechanical and of thermal trauma on gastro-intestinal functions are very different, and it is pointed out that in shock from different causes, certain manifestations may be quite different.

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# THE EFFECT OF DENERVATION ON THE FILTRATION RATE AND BLOOD FLOW IN DOG KIDNEYS RENDERED HYPEREMIC BY ADMINISTRATION OF PYROGEN

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The discovery that certain pyrogens, such as those found in typhoid vaccine (3) or in contaminated inulin (9), can cause a marked increase in renal blood flow in man, even when fever is prevented by an antipyretic, raises the question of the mode of action of these pyrogens. Are they humoral agents or is their action mediated through the nervous system? Since they do not have any immediate action on blood pressure or renal blood flow after intravenous injections (9), they do not fall in the category of common depressor or vasodilator agents. The investigations described below were designed to explore this question. They also contribute to the growing body of evidence indicating that the circulation of the kidney under normal conditions is not under tonic nervous control (9).

**METHODS.** The ureters of two dogs were explanted to the exterior by the method of Danilov as described by Grabfield (8), making possible the collection of urine from each kidney separately. Creatinine clearance, as a measure of the glomerular filtration rate, and diodrast clearance, as a measure of effective renal plasma flow (6), were determined under normal conditions and during the renal hyperemia induced by intravenous administration of 100 mgm. of the same lot of pyrogenic inulin (Pfanstiehl 268) used by Smith and his collaborators (9). In all these experiments the febrile reaction was blocked by a course of amidopyrine (four 5 grain tablets during the 12 hr. period before the experiment).

During an experiment the dog stood on a table supported in a sling. Diodrast and creatinine were administered by slow (0.8 cc. min.) intravenous infusion, and urine was collected by funnels placed under the ureter openings. Every effort was made to avoid obstruction of the ureters by pressure on the abdomen and all clearance periods in which the creatinine clearance showed marked irregularities which might have been due to obstruction have been discarded.

Creatinine concentration in cadmium filtrates (5) of plasma and in diluted urine was determined by the method of Folin (4). Diodrast concentration in cadmium filtrates of plasma and diluted urine was determined by the method of Alpert (1).

After a series of normal and hyperemic experiments the left kidney of each dog was virtually denervated by removing a section of the left sympathetic trunk. In dog 1 the trunk was removed from just posterior to the stellate ganglion in

the thorax to the second lumbar ganglion. In dog 2 the trunk was removed from just below the diaphragm to the second lumbar ganglion and in addition two splanchnic branches were located and interrupted by excising an inch or so. In both cases the interruption of the sympathetic trunk was verified at autopsy.

After the operation another series of control and hyperemic studies was carried out within six weeks to minimize the effect of regeneration.

**RESULTS.** There were no apparent physiological effects of denervation on renal function. The denervated kidneys behaved, in respect to urine flow, filtration rate, and blood flow, like the normal kidneys. In figure 1 the creatinine/diodrast clearance ratio (taken to be equal to the percentage of plasma filtered at the glomeruli) is plotted against the diodrast clearance ex-

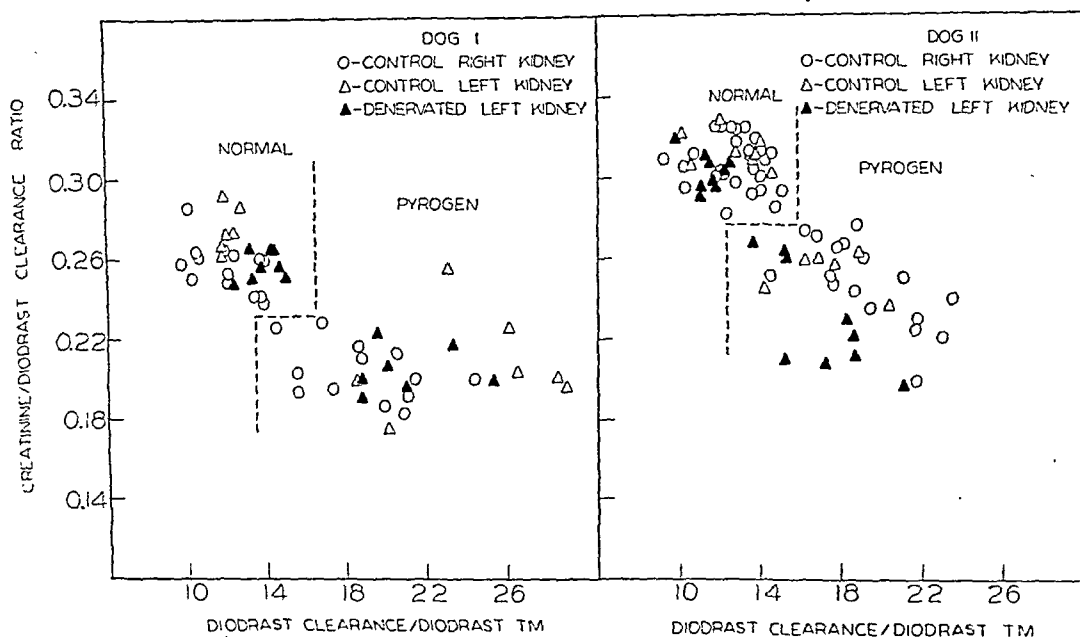


Fig. 1. The filtration fraction (creatinine/diodrast clearance ratio) in relation to the effective renal plasma flow (diodrast clearance) per unit of tubular excretory mass (diodrast Tm) in the intact and denervated kidneys of two dogs under normal conditions and after intravenous pyrogen. Each datum represents a clearance period.

pressed per unit of diodrast Tm. This latter is taken to be a measure of the effective renal plasma flow per unit of functional tubular tissue (6).

The correspondence of the denervated kidneys with the normal control values is close, both at normal and at hyperemic levels of renal blood flow. The denervated kidney of dog 2 showed a tendency toward a decreased blood flow which may be correlated with a partial obstruction of the ureter found at autopsy. Although the ureter and pelvis were expanded, histological examination of this kidney showed no signs of pathological change due to this partial obstruction.

In each dog one control experiment has been omitted from the chart because it was divergent from the other control experiments; in one case there was an anomalous hyperemia, in the other an unusually high filtration fraction.

DISCUSSION. The dog kidney responds like that of man and the seal (2) to inulin pyrogen, showing an increased renal blood flow and a decreased filtration fraction. Smith *et al.* (10) have postulated that this combination of events indicates dilatation of the efferent glomerular arterioles.

The fact that the denervated kidneys respond like the normal kidneys to inulin pyrogen indicates that the ultimate action of this agent is humoral in nature, but whether it exerts a direct effect by delayed action on the renal arterioles, or acts indirectly causing the secretion of some physiological vasodilating agent, cannot be answered by these experiments.

The failure of the denervated kidney to differ from the nondenervated control kidney in the same animal confirms the evidence cited by Smith in his review of this subject (10) that the renal nerves play no significant part in controlling the renal blood flow (or the urine flow) under basal conditions. As pointed out there, the contrary view was based chiefly on acute experiments in which the sympathetic nervous system had presumably been excited to some activity by anesthesia and traumatic procedures. Our observations are in agreement with those of Goldring *et al.* (7) on a single case of a human subject with unilateral sympathectomy, not only with respect to the effect of denervation but also the response to pyrogen.

#### SUMMARY

1. The filtration rate (as measured by the creatinine clearance) and the renal plasma flow, (as measured by the diodrast clearance) of the separate kidneys of two dogs with explanted ureters was determined under normal conditions and during the renal hyperemia caused by pyrogenic inulin.

2. Later one kidney of each dog was denervated by removal of a part of the sympathetic trunk on one side. The clearance experiments were repeated. No effect of denervation was observed.

3. It is concluded that the action of pyrogenic inulin in causing renal hyperemia does not involve the sympathetic nerves to the kidney.

4. It is demonstrated that in the quiet, unanesthetized, standing dog, there is no tonic sympathetic control of the renal vascular bed.

5. Denervation does not affect the urine flow.

We are indebted to Prof. H. W. Smith for suggesting the problem and for his valuable advice, to Dr. Philip Grabfield for explanting the ureters, to Dr. John H. Mullholland for performing the denervations and to Dr. Irving Graef for examining the animals at autopsy.

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# WATER INTOXICATION OF THE FROG (*RANA PIPPIENS*)<sup>1</sup>

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Former studies (5, 7) of water intoxication have been confined to mammals: cats, dogs, guinea pigs, rabbits, rats and man. An excessive amount of water introduced orally, rectally, subcutaneously, intravenously or intraperitoneally, regularly produces symptoms of intoxication, among which are asthenia, restlessness, frequent urination, diarrhea, nausea, retching, vomiting, frothing at the mouth, stupor, coma and convulsive seizure resulting in death. When a volume of water equivalent to one-third of body weight has been given, mild symptoms appear; and with two-thirds of body weight, pronounced toxic symptoms develop. Recovery follows if the administration of water is discontinued or if a hypertonic saline solution or an extract of the adrenal cortex is injected.

During this intoxication of mammals the water content of all organs shows a marked increase, that of the liver being the greatest. The plasma volume increases while the electrolytes of the blood, potassium, calcium, sodium, and chlorides decrease. The excreta of sodium chloride are in the vomitus and urine. All organs except the liver show a decrease in sodium chloride, that of the muscles is largest. The conclusion is that an ionic disturbance in the cells of the body is the basis of the mechanism of water intoxication.

In view of the remarkable toxicity of excessive amounts of water in mammals it seemed to us that the frog, due to the fact that it spends a large proportion of its life in water, might have developed a more adaptive water balance regulation and might yield different results when subjected internally to excessive amounts of water.

Males of the common green frog, *Rana pipiens*, in the winter condition were used throughout this investigation. During the first series of experiments the water was administered through a stomach tube. Usually a large portion of each administration was vomited immediately. That much was retained was evidenced by the results. Each animal received from 60 to 90 administrations of from 2 to 4 cc. of tap water more or less evenly distributed over 3 or 4 days. In spite of the fact that the frogs were given a relatively larger volume of water and over a longer period of time than were mammals, yet they survived.

A number of the animals were killed for examination. Gross inspection

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The authors are indebted to Prof. Ross A. Gortner, Jr. for helpful advice on chemical procedures.

showed the stomach and intestine distended with water and gas to approximately 2 to 4 times normal size; the liver was slightly enlarged, flaccid, and lighter in color; the kidney, enlarged and flaccid, and the body cavity filled with an abnormal amount of fluid.

Histological studies showed the liver sinusoids dilated, particularly in the vicinity of large central veins. The sinusoids were filled with erythrocyte "ghosts." Individual and groups of hepatic cells were swollen and often cloudy. There was no evidence of nuclear or cytoplasmic destruction.

In the kidneys Bowman's capsules were enlarged and not completely filled by the glomerulus. Tubules were somewhat distended, while the intertubular spaces contained erythrocyte "ghosts." The alimentary canal showed the effects of dilatation. The vessels of the submucosa contained abnormal amounts of blood. The blood erythrocytes were somewhat swollen without indication of hemolysis. No other organs and tissues showed appreciable change.

*Subcutaneous administration.* When it became evident that oral administration was ineffective in producing evidence of severe intoxication, subcutaneous injections were used. The periodic injection of 2 cc. of water into the large lymph sacs resulted in complete retention of the water. With this method water was given at the rate of 5 to 6 per cent of body weight at intervals of from 30 to 60 minutes until the total ranged from 230 to 400 per cent of the body weight. Workers with mammals usually found death to occur before as much as 100 per cent of the body weight had been administered. Even with the extraordinary amount injected subcutaneously none of the intact frogs died of water intoxication.

At no time were nausea, gagging or vomiting observed during this method of administration. As the injections proceeded the animals gave evidence of depression in a change of body posture and in curtailed activity. There were no convulsions but eventually many sank into a stupor.

Morphological and histological examinations revealed, except in the alimentary canal, which was not affected, conditions similar to those obtained with oral administration. The damage to the liver and kidney was more severe but similar to that of the former method. The liver cells appeared to have lost large portions of cytoplasm, while the nuclei and cell membrane remained intact. The Bowman capsules of the kidney were enlarged by from 2 to 3 times the normal size, often with a separation of the two layers of cells. A heavily staining material suggestive of hemolyzed blood often filled the capsule.

*Hypophysectomized frogs.* Since it is well known that the posterior lobe of the hypophysis plays a rôle in the control of elimination of water, it seemed reasonable to assume that water intoxication might prove to be more harmful to the hypophysectomized than to the normal animal. After some practice the removal of the gland was easily accomplished. Our criterion for the completeness of hypophysectomy was that established by Hogben and Winton (2). They found that complete removal resulted in a pale condition because of a maximal contraction of the melanophores due to the loss of hormone, while on the other hand partial removal rendered the animal decidedly dark.

After operation we allowed a period of from 7 to 10 days for recovery and then induced water intoxication in the frogs by hypodermic injection. The resulting symptoms were severe and somewhat similar to those reported as characteristic in mammals. For the first 20 to 30 cc. of water injected, no appreciable variation from the condition of the intact control frog was observed. However, when from 40 to 80 cc. of water had been administered, muscular tremor and twitching were noted. These were accentuated by handling. Later typical convulsions occurred. If then the injections of water were continued the animal became powerless and death ensued. On the other hand, when administrations were stopped at the onset of convulsions a slow recovery occurred that required as much as a week to 10 days.

*Chemical studies.* These included the determination of organ and body water, urine and organ chloride, and urine and blood calcium.

The following methods were employed. For water content the organs, after the animal was bled, were removed, laid in filter paper and rolled around to absorb adherent water, then dropped into weighing bottles and dried at 105 to 110°C. for from 36 to 48 hours.

Urine chlorides were determined by the Volhard-Arnold (10) titration method; body chlorides by a method worked out by R. A. Gortner, Jr., and tissue chlorides by the method of Van Slyke and Sendroy (9).

Sodium of the urine was observed by the micro-titration method of Kramer and Gittleman (3).

The urine calcium was estimated by the micro-titration method of Tisdall and Kramer (6) and the blood and urine calcium by the manometric determination of Van Slyke and Sendroy (8).

*Water balance.* The animal under observation was kept in a specially constructed urine chamber that was placed in a large moist chamber. This made it possible to follow the changes in body weight. When the frog was to be weighed and injected with water, it was first picked up and held over the funnel of the urine chamber while its own random movements caused the passage of urine. It next was weighed and then injected with distilled water. When injections were continued throughout a period of from 3 to 4 days, they were omitted during the night for from 8 to 10 hours. During the day the body weight rose steadily with the first 6 or 8 injections by between 10 and 25 per cent. With the first 3 or 4 injections practically no urine was passed. After this the elimination steadily rose until it approximately equalled the amount of water per injection. In the mornings, following the overnight opportunity for recovery, the body weight was found to be somewhat greater than the initial weight. Furthermore, the morning recovery weights were always slightly above those of the preceding day. Thus was indicated the establishment of a water balance at succeeding higher levels. This may be construed as an adaptation on the part of the frog to the excessive volumes of water. This fact was observed in all cases in which the experiment lasted 3 or more days.

When hypophysectomized frogs were used, the water balance was carefully investigated in view of the diabetes insipidus that has been produced experi-

mentally in mammals by hypophyseal operations. When the increase in body weight of the completely hypophysectomized frogs and that of the controls were plotted the curves were similar, which would not have been the case if the hypophysectomized animals had developed diabetes insipidus. No evidence of the polyuria of diabetes insipidus was present in any of our operated animals. Hence it is concluded that the problem of water balance was met in a similar manner by the two experimental groups.

That the water content of the two groups was practically the same is indicated by the water content of the brain, kidney, and liver (see table 1). These results, with the exception of those for the brain, are in accord with mammalian observations reported by Rowntree and others. Rowntree (5) reported that in a water intoxicated dog the intracranial pressure was increased by an amount equivalent to 35 mm. of water. He observed marked edema in the liver, portal veins, brain, and renal cortex.

It is concluded that, although the hypophysectomized frogs were more severely damaged by water injections than were intact animals, the water content of the two groups was similar. The curves of change in body weight and the output of urine of each group were surprisingly similar. An outstanding difference be-

TABLE 1  
*Water content, in percentage*

CONDITION OF FROG	BODY	BLOOD	BRAIN	KIDNEY	LIVER
Normal.....	79.1		83.2	82.2	70.8
Intact, H <sub>2</sub> O intoxicated.....	81.2	87.1	83.5	85.0	75.8
Hypophysectomized, H <sub>2</sub> O intoxicated...		87.9	83.6	84.5	76.5

tween the reaction of mammals when compared with the frog is that the latter maintains its normal content of water in the brain, while the former experiences edema of that organ.

*Urine chlorides.* All determinations were made for 10 cc. of urine. The normal samples were pooled urine from many frogs fresh from the tanks filled with running tap water which contained chlorides. The chloride content of urine samples taken at different times ranged from 0.5 to 0.9 mgm., average 0.73 mgm. These values were regarded as normal in that the conditions for intake were adequate. Samples of the urine of water intoxicated animals were secured as each animal remained in the moist atmosphere of the urine chamber, in which there was no opportunity for NaCl absorption. The samples were taken at irregular intervals as it was necessary to wait until an animal had urinated from 10 to 13 cc. The output per sample ranged from 0.5 to 7.4 mgm. (table 2). It seemed that during the progress of water intoxication the control of body chloride was at first broken down but that later there was an attempt to conserve a minimum compatible with life (see frog 27, table 2).

In order to test the above conclusion the entire carcasses of normal and water intoxicated frogs were analyzed for chloride. The results are given in table 3.

The intoxicated frogs after having received approximately 400 per cent of body weight of water by subcutaneous injection were allowed to dehydrate for 10 hours; then were killed, desiccated at 104 to 108°C., and dissolved in concentrated  $\text{HNO}_3$ . The chloride loss by the intoxicated frogs, W38 and W39, was 33.5 and 46.5 per cent respectively of the average content of normal animals. Hence it is evident that a considerable amount of chloride remained in the body of severely intoxicated frogs.

The chlorides of the urine of five hypophysectomized frogs were determined during the period of water intoxication. The content of 10 cc. samples of urine gave results that corresponded to those obtained with intact intoxicated animals. There was in three cases a distinct rise and fall in the chloride concentration

TABLE 2  
*Chloride concentration in 10 cc. of urine*

	I	II	III	IV
	mgm.	mgm.	mgm.	mgm.
W. 23	1.3	3.2	6.4	3.8
W. 27	1.1	7.4	1.5	0.7
W. 32	1.6	2.3	1.4	
W. 38	2.2	0.9	1.8	1.8
W. 39	1.9	2.0	0.7	0.5

TABLE 3  
*Chloride content of normal and water intoxicated frogs*

ANIMAL	BODY WEIGHT	DRIED WEIGHT	TOTAL CHLORIDES	DIFFERENCE FROM NORMAL AVERAGE	LOSS OF BODY CHLORIDE
	grams	grams	mgm.	mgm.	per cent
Normal 1.....	31.8	6.5	28.7		
Normal 2.....	31.0	6.6	27.5		
W.I. 38.....	34.7	6.5	18.7	9.4	33.5
W.I. 39.....	33.7	6.5	15.0	13.1	46.6

similar to frogs W.27 and W.39 in table 2. Hence it may be concluded that the loss of chlorides was similar in the two groups studied.

*Organ chlorides.* Misawa (4) subjected rabbits to water intoxication and studied the chloride losses in the various organs of the body. All organs, save the liver, showed a decrease. This ranged from 20 to 51 per cent. We determined the chloride content of the liver, kidney, and brain of frogs subjected to as much as 400 per cent of water. Each organ lost chlorides; the liver 31, the kidney 15.5, and the brain 10 per cent (see table 4). These results suggest that the frog does not release chlorides as readily as rabbits. In table 4 light and heavy intoxication are compared. It should be noted that the liver lost chloride under both treatments.

*Calcium.* Since the excretion of water and chlorides was not greater among the hypophysectomized than among intact frogs, it occurred to us that the

calcium ion might be involved in the production of convulsions. Charles (1) found that in an African toad, *Xenopus laevis*, the removal of either the anterior lobe alone or of both lobes of the pituitary body caused a persistent fall in serum calcium.

Tests for calcium in the urine of water intoxicated intact and hypophysectomized frogs were negative. Hence blood examinations were made of pooled blood of two or three animals. Removal of the pituitary gland of itself was found to cause a slight fall in blood calcium (table 5). Water intoxication reduced the blood calcium of intact animals from an average of 7.7 to 6.9 mgm. per 100 cc. of blood. In water treated hypophysectomized frogs the average

TABLE 4

*Organ content of chlorides of normal and water intoxicated frogs, in milligrams per gram of organ*

CONDITION OF ANIMALS	BRAIN	KIDNEY	LIVER	MUSCLE
Normal.....	7.78	8.61	4.04	2.23
Light W.I.....	7.65	7.70	3.72	
Heavy W.I.....	6.98	7.26	2.79	1.96

TABLE 5

*Calcium content of whole blood of frogs in milligrams per 100 cc.\**

INTACT		HYPOPHYSECTOMIZED	
Normal	Water intoxicated	Normal	Water intoxicated
7.2	6.8	5.9	5.7
7.4	6.1	7.4	6.6
8.1	8.0	6.5	6.6
7.4	6.2	7.0	5.5
8.2	7.2		5.7
			5.3
Average... 7.7	6.9	6.7	5.9

\* Each determination required the blood of 2 or 3 frogs.

was 5.9 mgm. In 12 of 18 animals it was 5.7 mgm. or less. Waggener (11) observed that after removal of the parathyroids in frogs a drop occurs in the blood calcium which is equivalent to about  $\frac{1}{4}$  to  $\frac{1}{3}$  of the total calcium normally present. In our water intoxicated hypophysectomized animals the drop ranged from 15.4 to 32.1 per cent. The drop in calcium may, therefore, explain the developemnt of convulsions among water intoxicated hypophysectomized frogs.

#### SUMMARY AND CONCLUSIONS

Frogs in the winter condition withstand water intoxication better than mammals. Water administered to the normal animal by stomach tube or subcutaneously at intervals of 30 to 60 minutes in doses of 2 to 4 cc. over a period of 3 days failed to kill them.

Histological studies revealed severe damage in the kidney and liver, but this was not permanent. Complete recovery occurred within 10 to 14 days.

Hypophysectomy renders frogs susceptible to water intoxication. When they have received from 40 to 80 cc. of water, muscular tremor and twitching appear. If injections are then continued convulsions occur and they die.

The water content of the body and organs of the intact and hypophysectomized water intoxicated frogs rose approximately equally. The brain did not show hydration.

The excretion of chlorides during water administration at first rose but later decreased. It is suggested that this is an attempt by the body to conserve a minimum compatible with life.

The calcium content of the blood in water intoxicated frogs was lower in the hypophysectomized than in the intact animals. In the water intoxicated hypophysectomized frogs the drop in blood calcium often was as large as a third of the normal content. Since others have reported that a drop of from  $\frac{1}{4}$  to  $\frac{1}{3}$  of the total blood calcium causes tetanus in parathyroidectomized frogs, it is suggested that the similar drop in water intoxicated hypophysectomized frogs also causes convulsions and death.

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# THE RESPIRATION OF NEURONES<sup>1</sup>

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A considerable literature now exists dealing with the gross respiration of the central nervous system, and evidence is accumulating on the respiration of structurally delimited regions (Gerard, 1938). Experiments of the latter type are mostly indirect, metabolic intensity being inferred from vascularity (Wolff, 1938; Craigie, 1941; Scharrer, 1939), resistance to anoxia (Sugar and Gerard, 1938; Kabat, Dennis and Baker, 1941; Fazekas, Alexander and Himwich, 1941; van Harreveld, 1941) or hypoglycemia (Tyler, 1940; Gellhorn, Packer and Feldman, 1940), concentration of oxidizing enzymes (Campbell, 1939), rates of reaction with special oxidation systems (ferric chloride, Gerard, 1938; ferricyanide, Michaelis and Quastel, 1941), etc. Also, in most such studies, the findings are at best applicable to considerable masses of cells mixed with other elements.

Many problems of neural function cry for more precise knowledge of the respiratory intensity per neurone or even neurone part. The present study, based on parallel measurement of oxygen consumption and cytoarchitecture of small bits of frog brain, is a step towards securing such knowledge.

**METHODS.** 1. *Tissue.* A frog was decapitated, the sides of the head snipped off, and the top of the brain case removed with forceps. The brain was carefully lifted on to a paraffin plate under a dissecting microscope and the meninges peeled off. Injured brains were discarded. A slice including the desired region was obtained by transections anterior and posterior to it, and then the particular region was cut out. A minimum of Ringer's solution (no substrate) was used. This was blotted off before weighing the tissue bit, which was remoistened in the capillary. Variations in  $QO_2$  values (per gram moist tissue) are partly due to the difficulty of obtaining constant blotting, and proper weights, of tissue bits weighing but a milligram or two. From decapitation to completed dissection was never as much as five minutes, and respiration readings could be begun by another 5 to 10 minutes.

The structures chosen were of widely varied origin and architecture—the Anterior Olfactory Nucleus (AON), the Hippocampus (H), the Primordium Pallii (PP), the Ventral Hypothalamus (VT), and the Cerebellum (C) (fig. 1). AON composes the dorsal half of a slice obtained by a section at the junction of the olfactory bulbs and hemispheres and one a millimeter caudal. The cells in this nucleus receive fibers from the olfactory tract and send processes to the hemisphere. H constitutes the dorso-medial segment of the ring-shaped slice

<sup>1</sup> A preliminary report of this work appeared in *This Journal*, 129: 445, 1940.



obtained from the middle third of the hemisphere. It is sharply delimited by gross landmarks, is large and relatively homogeneous, and is obtained with minimal sampling error. It represents the most highly organized brain region in the frog. PP is lateral to H, on the crest and lateral wall of the hemisphere with superficial white matter and a periventricular cell sheet. Since samples were often obtained by a simple horizontal slice from the top of the hemisphere, when speed was at a premium, this region was less precisely dissected than the others. VT bulges out from the ventral brain surface, the pituitary having been removed with the meninges. It is easily cut from a slice which includes the hypothalamus and contains considerable white as well as grey matter. C is the dorsal portion of the slice taken between the optic lobes and the 4th ventricle, and contains about half white matter. Since C is largely separate in situ, a minimum of damage is produced by cutting in this case.

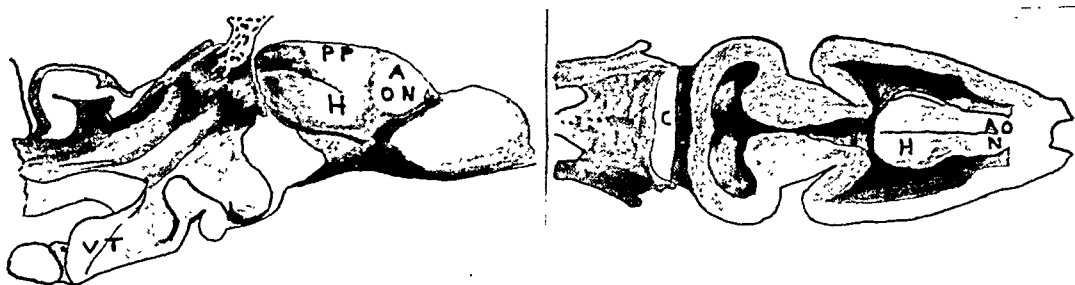


Fig. 1. Medial view of sagittal and view from above of horizontal section of the frog's brain, indicating the regions studied. Modified from "Handbuch der vergleichenden Anatomie der Wirbeltiere" and from Kappers-Huber-Crosby, Comparative Neurology, respectively.

2. *Respirometer*. The capillary respirometer (Gerard and Hartline, 1934) was used, with some modification, to measure oxygen consumption. To hasten temperature equilibrium, the instrument was placed vertically and the outer container filled with Ringer's solution except for 20 cc. of air trapped under inverted smaller tubes in it. Two thin-walled capillaries, about 2 sq. mm. cross section (later measured exactly in each case) and 200 c. mm. total volume, were carried on a lucite rod, which passed through the stopper of the outer vessel. Their upper ends were sealed with paraffin after inserting two filter paper strips and wetting them with isotonic KOH and  $H_2SO_4$  respectively; tissue on a cellophane tray was placed through the other end of one, the second served as a control. All parts of the instrument were brought to temperature ( $24^\circ C$ ) in the thermostat before the capillaries were mounted, and then placing the rod and stopper forced Ringer's solution a few millimeters into the capillaries, to serve as index fluid. Movements of the menisci, illuminated from behind with green light, were followed with an ocular micrometer in a long focus microscope. Some sticking of the menisci occurred and vibrating the holder with a buzzer for 5 sec. before each reading improved the regularity of readings.

One micrometer division corresponded to a volume of 0.0016 cu. mm., but ir-

regularities rendered short interval readings of little value. (See, however, further improvements by Tobias and Gerard, 1941.) Control and experimental tubes were read at alternate half minutes, and a sliding 3 min. average of the control was subtracted from a similar average of the tissue tube. In obtaining absolute  $QO_2$  values for each tissue type, the final 30 min. readings of each run were averaged.

3. *Evaluation of cells.* Respiration values per cell are so far available only on naturally separate cell species, such as blood or sex cells or unicellulars. Counts on histological sections are usually impracticable because of irregular distribution and large numbers—about 100,000 per mgm. of brain. A possible solution is to macerate the brain sample after determining its respiration, and to count and measure the cell nuclei thus brought into homogeneous suspension.

The older histologists made frequent use of macerating solutions. Recently Crossman (1937) (also Stoneberg, 1939) has used 5 per cent citric acid to isolate muscle nuclei, and Isaacs (1937) and Farrar (1936) have removed connective tissue from bone marrow with serum. Zirkle (1928, et. seq.) has shown that, while basic fixatives destroy nuclear structures, acid ones preserve the nuclear chromatin. This is especially true of acetic acid which, because of rapid penetration, dominates in any mixture containing it. Further, he found that acid, per se, does not affect the size of cell nuclei, the usual shrinkage being due to dehydration.

In the present experiments the tissue bit was taken from the capillary, usually one hour after the decapitation, and gently triturated for 2 min. (in a 5 cc. glass test tube with a glass pestle ground to fit) in a measured volume—at least 20 times the tissue mass—of 25 per cent acetic acid in water. This dissolved the cytoplasm but left the nuclei intact in suspension. The suspension was further diluted with nine volumes of a 0.1 per cent solution of gentian violet in distilled water, to stain the nuclei. After thorough mixing, the diluted suspension was drawn into a red cell diluting pipette, mixed for an additional two minutes, and a drop placed in the hemocytometer counting chamber for counting. Two hundred to 1000 cells were counted in each sample. In calculating the cells per milligram (cells per cubic millimeter in the final suspension, times total volume in centimeters of acid and dye solutions, divided by sample weight in milligrams), the volume of the brain sample was neglected.

Several possible sources of error have been explored. That maceration and mixing were adequate was shown by the reproducibility of cell counts on separate drops of the same tissue suspension. Independent duplicate counts, two on each of three brain regions, gave a maximum variation between two drops of 7 per cent, an average of 4 per cent. A more severe check, testing in part the maceration and nuclear destruction as well as the reproducibility of the entire procedures of suspension and counting, was to compare cell counts on bilateral brain samples. Tests on three brain regions gave differences of 4, 17 and 21 per cent—well within the error of exact dissection and weighing of the tissue bits.

Further tests were made with frog's nucleated red cells, by comparing nuclear counts with each other and with cell counts made with Hayem's solution as

diluent. Blood was diluted 22 times with acetic acid (in the white cell counting pipette), the liquid expelled and "ground" for 2 min., diluted a further 10 times with gentian violet, and enumerated. Another sample of the same blood was merely diluted twice (1:22 and 1:10) with Hayem's solution. In three tests, on different bloods, the counts were, in thousands per cu. mm., 549 and 560, 582 and 596, and 749 and 741 in acetic acid and Hayem's solution respectively—an average deviation of 1.7 per cent. When one blood sample was directly diluted (1:200) with Hayem's solution and another portion weighed, "ground" with acetic acid, etc., the counts differed by as much as 14 per cent. These results prove that red cell nuclei are not destroyed by the maceration procedure used and that, except for the weighing of the sample, the whole method is valid to within 5 per cent. Since counts on bilateral regions agreed as well as the weigh-

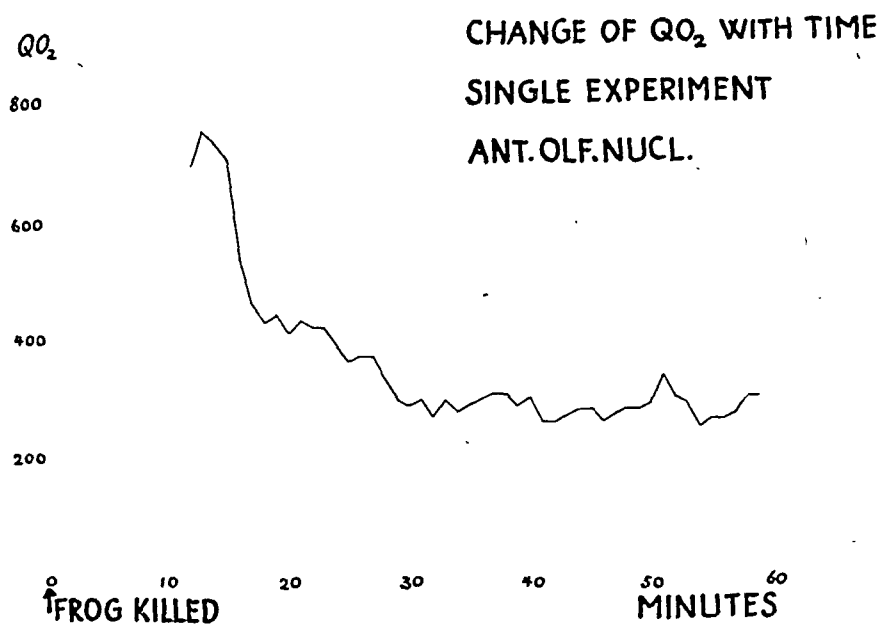


Fig. 2. Single experiment, illustrating reading variability

ing error permits, it is almost certain that no significant nuclear destruction occurred in these cells either.

The problem of what cells—neurones, neuroglia, endothelium, erythrocytes—are represented by the nuclei counted, and the related one of particular sizes and shapes of the nuclei from particular brain regions, will be considered later.

**RESULTS. 1. Respiration.** Seventy-seven oxygen consumption curves were obtained from the 5 brain regions and the sciatic nerve. Figure 2 reproduces a single curve and table 1 gives average values for each tissue. Decapitation is taken as zero time in all curves and averages. All brain regions, but not the sciatic, showed initially rising values, to a maximum at 15 to 20 min., and then a decelerating fall for the remainder of the hour.<sup>2</sup> For all quantitative com-

<sup>2</sup> The initially low readings are the result of an instrumental error, perhaps an initial lag in  $CO_2$  absorption. When one bilateral brain bit is mounted in a capillary as usual, and the other after standing for 20 min. or more on the cellophane tray, the second shows the same

parisons, the average value of the fairly stable respiration of the last half-hour was used. This was but little greater than the value for the final 5 min.

The respiration of frog nerve (Gerard, 1930) and brain (Rosenberg, 1935) averages over 60 per cent higher in summer animals than in winter ones. Similar differences appeared in these experiments, although most of them were performed in the winter. The results with PP, however, were mostly obtained in the summer and cannot be directly compared with other values.

Results are summarized in table 1, which also includes the statistical estimate of their quantitative reliability. Winter values for brain  $QO_2$  range from about 560 to 730 at the maximum and 390 to 470 in the final half-hour, about 8 times that of the sciatic; and summer values are higher. Yet even these are con-

TABLE 1  
*Mean respirations of various regions of frog nervous system*

TISSUE	NUMBER OF FROGS USED	MEAN $QO_2^*$ AT MAXIMUM	MEAN $QO_2$ FOR LAST 5 MIN. (1 HR. AFTER DECAPITA- TION)	MEAN $QO_2$ FOR LAST 30 MIN. AFTER DECAPITA- TION	$\sigma x \dagger$ (FOR LAST 30 MIN.)	$\sigma \bar{x} \ddagger$ (FOR LAST 30 MIN.)
Hippocampus.....	10	680	440	475	$\pm 115$	$\pm 7$
Cerebellum.....	10	725	355	400	$\pm 130$	$\pm 8$
Ant. Olf. Nucl.....	10	685	350	390	$\pm 165$	$\pm 7$
Hypothalamus.....	6	565	370	395	$\pm 155$	$\pm 12$
Sciatic nerve.....	10	90	60	65	$\pm 30$	$\pm 2$
Primordium Pallii.....	10	1055	610	675	$\pm 735 \S$	$\pm 50$

\*  $QO_2 = \text{mm}^3 \text{O}_2/\text{hr}/\text{gm wet weight}$ .

$\dagger \sigma x$  (standard deviation of the readings) =  $\sqrt{\frac{\sum (x - \bar{x})^2}{N}}$ , where  $x$  is a single reading,

$\bar{x}$  the mean,  $N$  the number of readings. Over two-thirds of the readings normally fall in the range,  $\bar{x} \pm \sigma x$ .

$\ddagger \sigma \bar{x}$  (standard error of the mean) =  $\frac{\sigma x}{\sqrt{N}}$ . The chances of the observed  $\bar{x}$  being more than  $3\sigma \bar{x}$  from its "true" value are less than 3 in 1000.

$\S$  The great variability of primordium pallii readings is due to sampling conditions. In many of these experiments a very rapid removal was the prime consideration and constant anatomical bits were not obtained.

low initial readings as had the first. Thus, in seven paired tests on PP (about 1.5 mgm. each) readings on the first samples averaged 1000 9.5 min. after decapitation, rose to 1400 4 min. later, and had fallen to 765 at 25 min. The second samples were mounted after half an hour and readings averaged 450 9 min. later, rose to 740 in another 5 min., and had fallen to 520 at the end of the run, one hour from decapitation. Clearly, except for the initial low period, the respiration of the second sample is a continuation of that of the first. The maximum values may, correspondingly, be somewhat excessive due to an "overshoot." This seems unlikely in view of the paired runs discussed and of the extensive evidence of a progressive fall of brain respiration after isolation (see Gerard, 1938), but is another reason for our using the later respiration values for quantitative evaluation. It is also worth noting that the high values during the first half-hour prove that oxygen diffusion into the tissue is not a limiting factor. This was further tested by the use of larger brain bits, which gave the same  $QO_2$  values as smaller ones.

siderably above those of Rosenberg (1935), in the range 150 to 300, and of Ashford and Holmes (1931), 330 for intact and 250 for chopped brain; they are of the same order as those of Bass (1923), 415. Spring values for these same brain regions, obtained under similar but not identical conditions (Tobias and Gerard, 1941) average some 20 per cent higher. The maximal values here reported are probably more indicative of the *in vivo* rates than are the later ones; the lower values of other workers may be due to their working later after dissection and with whole brain, which contains a higher proportion of white matter than do our regions. The early high values are not due to injury—compare C, with only one cut surface, to AON with four—nor to an oxygen debt, for any possible inadequacy of oxygen could have lasted 3 to 5 min. at most. Conversely, the later low values are to be expected from substrate depletion and possible loss of a serum factor (Brookens, Ectors, and Gerard, 1936; Schaffer, Chang, and Gerard, 1935).

H has a higher respiration rate than the other brain regions, and is to be regarded as a "higher" center. Other direct *in vitro* respiration measurements on pigeon (Gavrilescu and Peters, 1931) and ox (Dixon and Meyer, 1936) (see also Quastel, 1939) offer interesting comparisons. The  $QO_2$  of pigeon cerebellum, 1030, is 2.60 times that of the frog; and the cerebrum  $QO_2$ , 1260, is 2.65 times that of the frog's hippocampus. Ox cerebral cortex, 1700, ammon's horn, 1260, and thalamus, 1170,  $QO_2$  values are respectively 3.6, 2.7, and 2.9 times as great as those of the most equivalent frog structures. Evidence from anoxia (Sugar and Gerard, 1938; Hermann, et al., 1939) and hypoglycemia (Tyler and Ziskind, 1940) also indicates a parallelism between metabolic intensity of a brain region and its position along the neuraxis and in evolution.

**2. Cell population.** Figure 3 reproduces photomicrographs of characteristic fields obtained after maceration and staining of each of the brain regions. Although some debris is always present, the nuclei stand out as stained units containing chromatin structures and with a smooth unbroken boundary, the nuclear membrane, clearly visible. Only these were counted and measured; and the absence, or constancy, of any personal factor of selection is indicated by the degree of statistical reliability of the results.

It is apparent at once from figure 3 that the cell populations of the different brain regions are fairly unique for each, as judged by nuclear sizes and numbers. Similarly, the average cell count per hemocytometer square (0.1 cmm.) was 105 for C, 70 for AON, and 27 for H. The results of the cell counts are presented in table 2. Despite considerable variation from brain to brain, the count for each region centers around its own characteristic value, which is significantly different from that of others.

For a more exact analysis, and to distinguish between kinds of cells, nuclear dimensions were measured with an ocular micrometer. The greatest diameter and the maximum width at right angles to this were obtained on 100 consecutive nuclei, as located across the counting chamber, of each preparation. Since 10 such preparations of AON, H, and C were studied, a composite picture of 1000 nuclei was obtained for each of these regions.

Nuclei of blood cells were obtained by applying the maceration technique to whole blood, and the dimensions of 300 nuclei, from 3 samples, measured as above. To identify endothelial cell nuclei in the brain bits, the trituration in acid was stopped early, in half a minute, and the imperfectly macerated tissue then stained as usual. Scraps of capillary endothelium were thus preserved and their nuclei identified. Three hundred of these were measured. The dis-

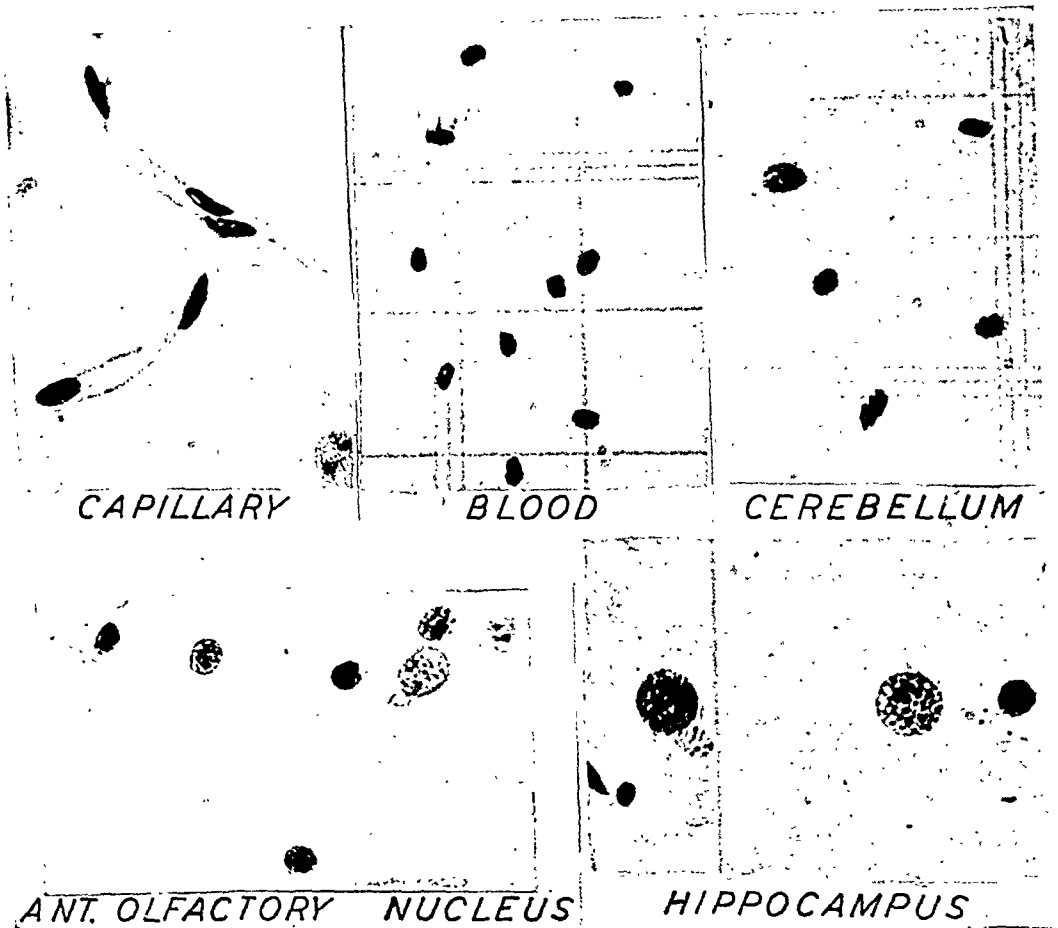


Fig. 3. Photomicrographs of characteristic fields obtained after maceration and staining of nuclei. Five-hundredths millimeter between squares on the R.B.C. field gives magnification.

crimination of glial and neurone nuclei was not satisfactory.<sup>3</sup> These cell types will, therefore, be considered together.

Although fixation shrinkage and distortion would not affect relative measurements of the different cell nuclei, absolute values might be seriously off. To check this, red cell nuclei were measured after treatment with Hayem's solution and with acetic-gentian violet and the results compared. Table 3 shows clearly

<sup>3</sup> Using toluidine blue, instead of gentian violet, Doctor Tobias finds the glial nuclei to stain with a more greenish tinge than those of neurones, but the conditions for obtaining this differentiation have not been worked out.

TABLE 2

*Total cells per milligram for each tissue (multiply all figures by 10<sup>3</sup>)*

EXPERIMENT NUMBER	BLOOD	HIPPOCAMPUS*	CEREBELLUM	ANTERIOR OLFACTORY NUCLEUS	HYPOTHALAMUS	WHOLE CENTRAL NERVOUS SYSTEM
1	640	60	215	175	145	61
2	560	49	205	115	100	
3	550	46	250	190	175	
4	550	48	260	90	145	
5	580	49	235	90	155	
6	595	42	260	135		
7	750	55	315	75		
8	740	45	165	145		
9	690	62	200	105		
10	610	67	170	120		
$\bar{x}$	627	52	228	124	143	
$\sigma x$	69	8	43	36	25	
$\sigma \bar{x}$	$\pm 26$	$\pm 3$	$\pm 16$	$\pm 13$	$\pm 14$	

\* The more consistent figures for hippocampus than for other regions are undoubtedly the consequence of better sampling. Because of the few large cells, more drops of suspension were counted. Had a similarly extensive sampling been used in the other cases a corresponding improvement in reliability should have resulted.

TABLE 3

*Comparison of nuclear dimensions in maceration fluid and in Hayem's solution*

	METHOD OF PREPARATION	
	Acid and gentian violet	Hayem's solution
	100 cells	50 cells
Surface:		
Average.....	144	150
$\sigma x$ .....	29	33
$\sigma \bar{x}$ .....	$\pm 2.9$	$\pm 4.6$
$P$ .....	0.12	
Volume:		
Average.....	135	140
$\sigma x$ .....	41	24
$\sigma \bar{x}$ .....	$\pm 4.1$	$\pm 3.4$
$P$ .....	1.4	

Statistical analysis of the measurements on red cell nuclei prepared in Hayem's and in acid and gentian violet. Surface was calculated, for convenience, by the formula of  $S = \pi dl$ , where  $d$  and  $l$  are the short and long diameters respectively. Volume is calculated by the formula  $V = \frac{\pi}{6} d^2 l$ .  $P$  is the probability that the two samples are from the same universe. As  $P$  is much greater than 0.05 this identity can be taken as established.

that nuclei prepared by both methods fall into a single population; that is, the two treatments are equivalent. This finding is in agreement with Zirkle's results.

A further test was made by comparing nuclear dimensions of bilateral bits from the same brain. In figure 4 the abscissa represents the long nuclear diameter, the ordinate the short one, and each nucleus is represented by a dash within the one micron square to which its measurements relegate it. Since nuclei from one side are shown as horizontal dashes and those from the other as

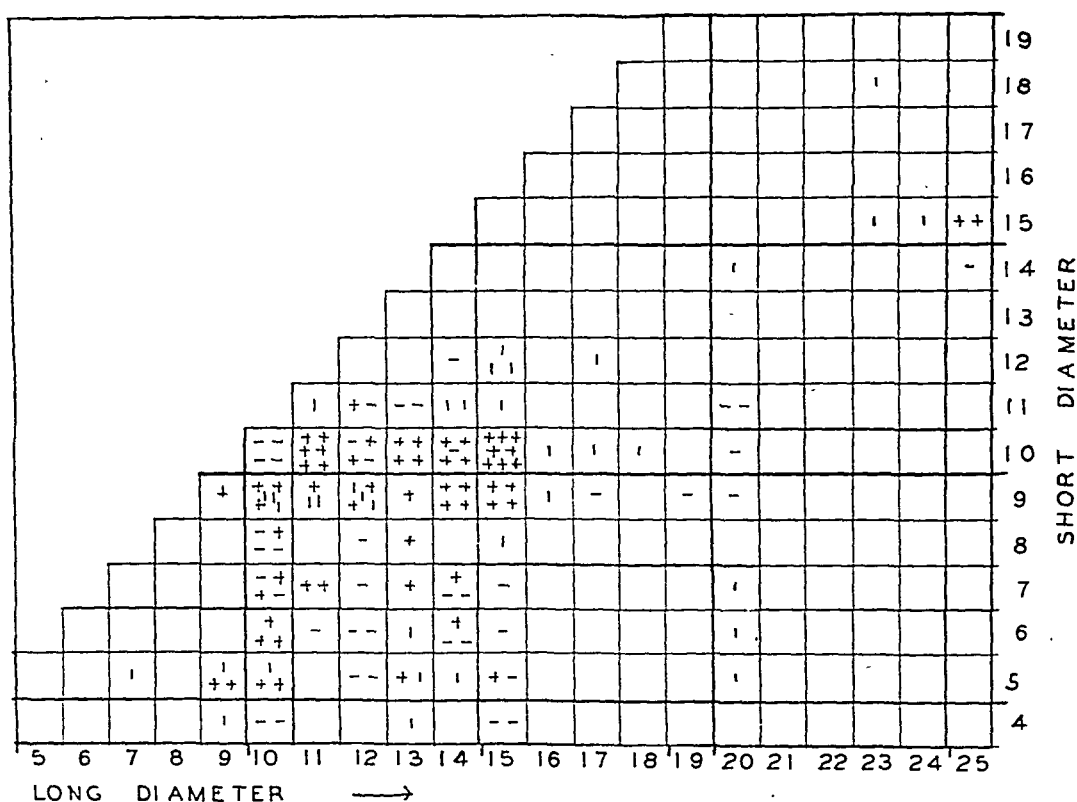


Fig. 4. Chart showing distribution of nuclear dimensions from like bilateral samples of one brain. The long and short diameters are indicated in microns. Each dash represents one nucleus; horizontal ones from one side of the brain, vertical ones from the other.

vertical ones, the crosses at once indicate the high degree of coincidence of the two sets. A similar amount of overlap was obtained when some nuclei in a given suspension were measured at once after preparation and others 24 hours later. Finally, measurements made on nuclei in fixed (formol acetic) and stained frog brain sections were compared with those on macerated preparations (we are indebted to Dr. M. L. Silver for values on one such set).

Rough long and short nuclear diameters, in sections and suspension respectively, are: red cells, 8 x 4 and 10 x 5; endothelial cells, 12 x 8 and 15 x 7; H, 12 x 12 and 17 x 17; C, 8 x 6 and 10 x 10; AON 10 x 6 and 15 x 10. Blood and



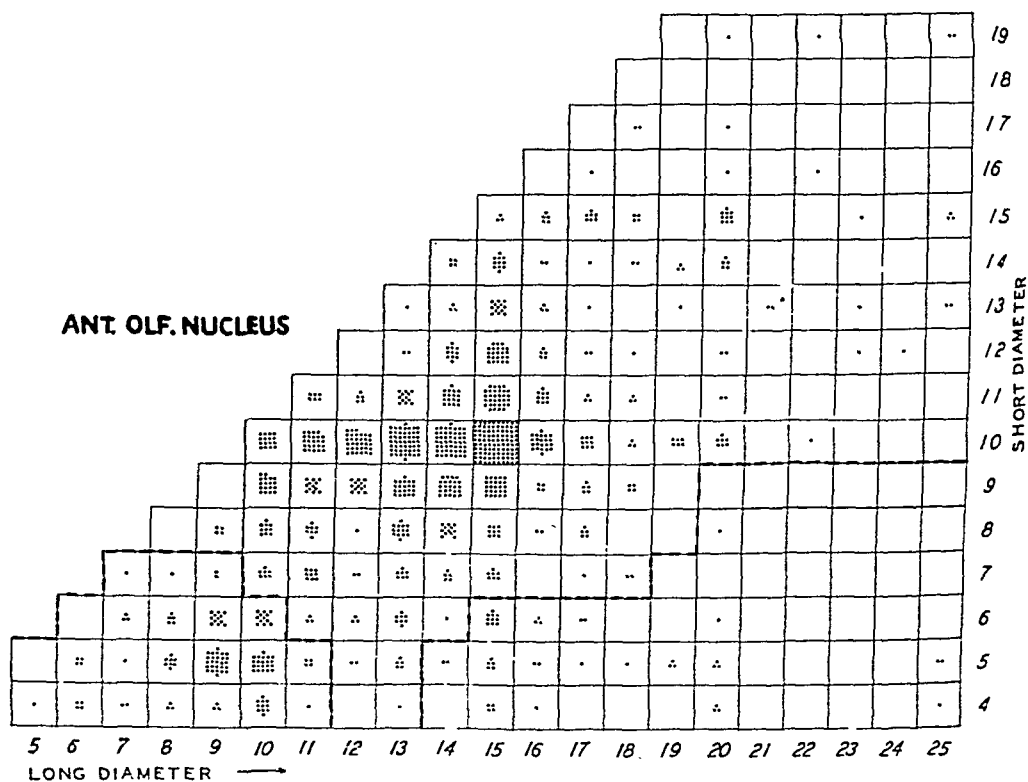
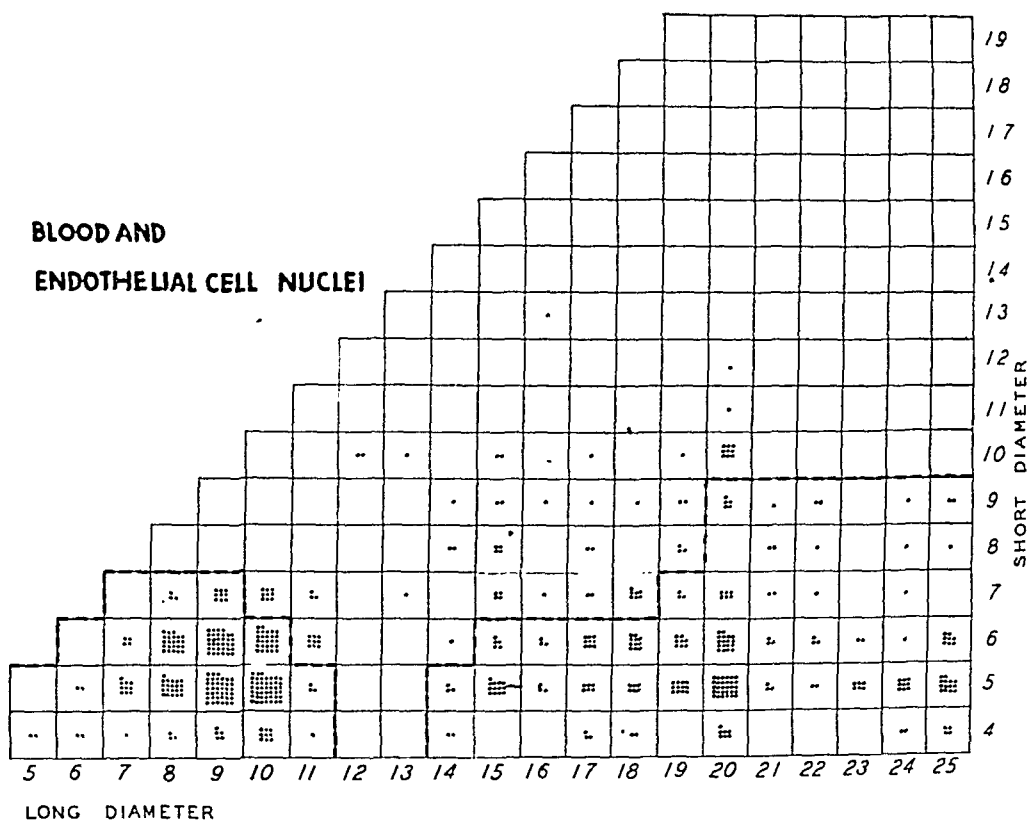


Fig. 5. Charts showing the distribution of nuclear dimensions in various tissues. Each dot represents one cell nucleus of the indicated dimensions in microns. The blood and endothelial cells were measured in separate preparations. The dotted lines in the brain charts are drawn to include 90 per cent of these non-neural cells.

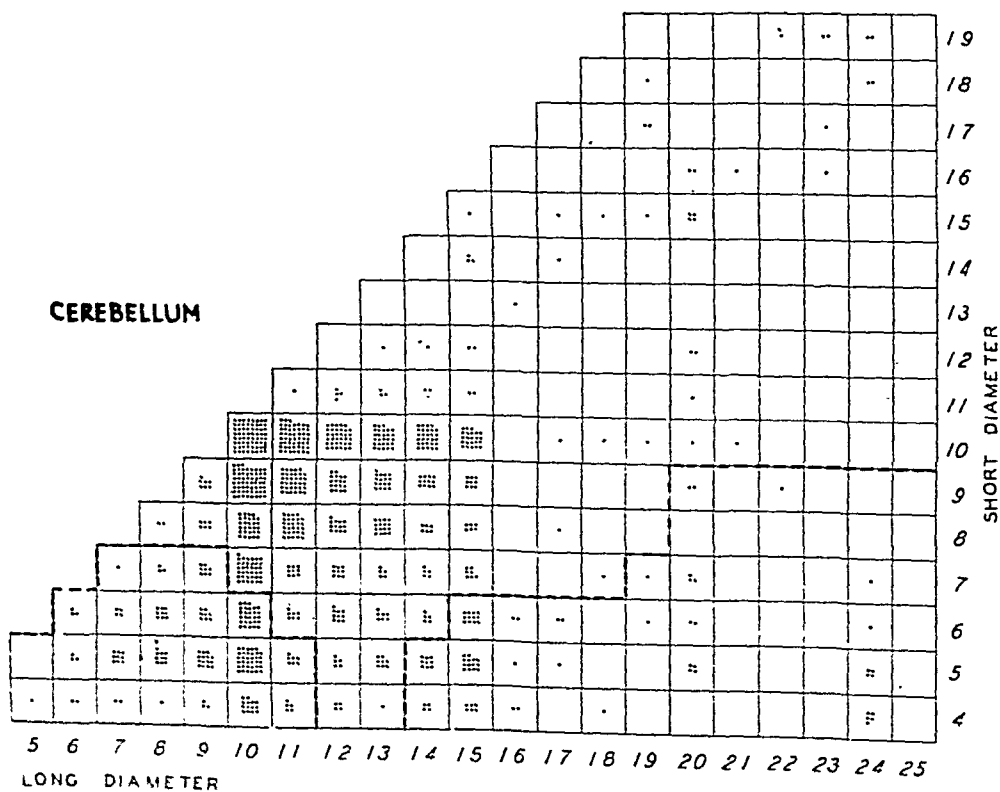
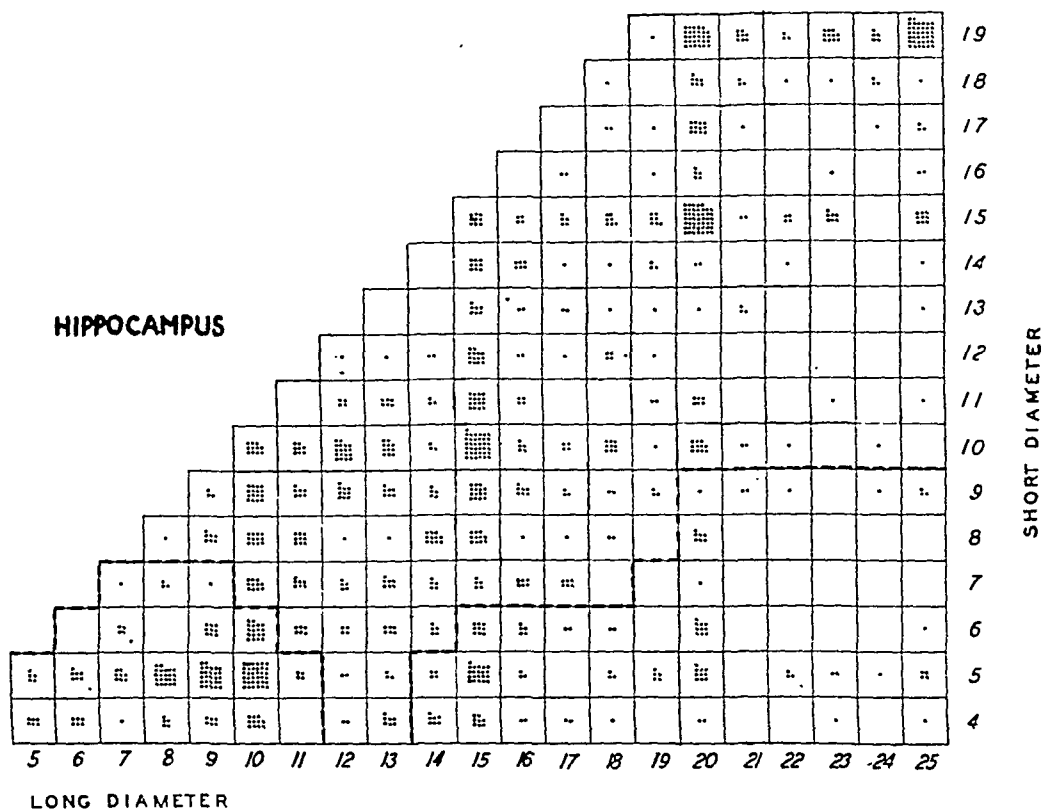


Fig. 5  
59

capillary cell nuclei show fair agreement, the fixed preparations being somewhat larger, while the discrepancy with nuclei of neural elements is greater. Since technical errors also appear in section measurements and since a large sampling error is probable (only a few dozen nuclei were measured), it is hard to evaluate this divergence; but obviously the absolute dimensions we have used are open to some question. For a doubling of radii, values per unit surface would be low in absolute units by a factor of four; values per unit volume, by a factor of eight. Fortunately, even if all neural nuclei are swollen in our suspensions, the various relationships will still hold, as between the different brain regions, if the swelling be proportional to true size—a reasonable assumption.

Charts showing the distribution of nuclear dimensions for the three intensively studied brain regions and for erythrocytes and endothelial cells are presented in figure 5. That these differ is patent. Cerebellar nuclei are small and rather homogeneous, centering about  $18 \times 10 \mu$ ; those of AON are more variable and center about  $10 \times 15 \mu$ ; while those of H are quite scattered and run to much larger dimensions.

It is necessary to correct the brain regions by excluding blood and endothelial nuclei in order to deal only with neural elements. This is difficult because of some overlap in dimensions of nuclei of these different types; but it is none-the-less possible to make an adequate separation. An area was blocked off on the nuclear dimension charts of blood and of endothelial cells so as to include 90 per cent of all nuclei in each case. The same areas were then marked on the charts for each neural region and the nuclei in them excluded from the final count. Thus, presumably, the neural count includes some 10 per cent each of the other cell types and excludes an unknown portion of neural elements. Actually, the overlap of dimensions is small enough and the percentage of non-neural nuclei in the suspension low enough so that varying the areas subtracted, to include 100 per cent or 80 per cent of the non-neural nuclei instead of the usual 90 per cent, alters the neural cell count by only 2 to 3 per cent. The error in evaluating blood and endothelial cells is, of course, greater. Further refinements should, however, make it possible to estimate the relative capillary length and the blood supply<sup>4</sup> for each brain region from such nuclear studies. Table 4 gives the cell relations as obtained by our present procedure.

3. *Nuclear dimensions.* Aside from their value in distinguishing cell types, the nuclear diameters permit calculation of other nuclear dimensions. Assuming an ellipsoid shape of the nucleus, the third, unmeasured, diameter was taken equal to the shorter measured one. Nuclear volume was then obtained from the formula  $V = \frac{\pi}{6} d^2 l$  ( $d$  = short diameters,  $l$  = long one). Nuclear surface was calculated, for convenience, in terms of a cylinder, of length  $l - d$ , capped by hemispheres, of diameter  $d$ .  $S = \pi dl$ . For other comparisons, nuclear volume

<sup>4</sup> For example, from the known red cell count and the average number of red cell nuclei per milligram of brain tissue, it can be calculated that blood constitutes over 2 per cent of the brain volume, despite the free hemorrhage permitted on decapitation. Compare with the value of 8.3 per cent for the dog's brain (Weil, Zeiss and Cleveland, 1931).

squared ( $V^2$ ) and to the three halves power ( $V^{3/2}$ ) have also been calculated. These four values were calculated for each set of nuclear dimensions. To obtain, say, total nuclear volume,  $\Sigma V$ , per cubic millimeter of any brain region, the

TABLE 4

Percentage and absolute numbers per milligram of red blood cells, capillary cells, and nerve and glia cells in each tissue. (Calculated from fig. 5)

TISSUE	NERVE AND GLIA CELLS	RED BLOOD CELLS	CAPILLARY CELLS	TOTAL CELLS PER MGM.	RED BLOOD CELLS PER MGM.	CAPILLARY CELLS PER MGM.	NERVE AND GLIA CELLS PER MGM.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>				
Hippocampus.....	67	17.5	15.5	52,300	9,000	8,000	35,000
Ant. Olf. Nuc. ....	80.5	13	6.5	123,400	15,000	8,000	100,000
Cerebellum.....	70	19	11	227,600	43,500	25,000	160,000

TABLE 5

Calculated functions of the nuclear dimensions of nerve and glia cells

FUNCTION	CELLS	HIPPOCAMPUS	CEREBELLUM	ANT. OLF. NUCL.
Average function per cell, in microns				
Nuclear surface ( $\mu^2$ ).....	Total	405 $\pm$ 13*	275 $\pm$ 4	380 $\pm$ 4
	"Nerve"	505	315	420
Nuclear volume ( $\mu^3$ ).....	Total	935 $\pm$ 45	395 $\pm$ 14	585 $\pm$ 14
	"Nerve"	1290	495	635
(Nuclear volume) <sup>2</sup> ( $\mu^6$ ).....	Total	2,950,000 $\pm$ 210,000	325,000 $\pm$ 40,000	539,000 $\pm$ 35,000
	"Nerve"	4,350,000	445,000	654,000
(Nuclear volume) <sup>3/2</sup> ( $\mu^4$ ).....	Total	45,500 $\pm$ 2,900	10,000 $\pm$ 720	17,000 $\pm$ 700
	"Nerve"	66,000	13,500	20,000
Average function per milligram of tissue, in microns ( $\times 10^{-6}$ )				
Nuclear surface.....	Total	21.5 $\pm$ 1.4	62.5 $\pm$ 4.5	46.5 $\pm$ 5.0
	"Nerve"	17.5	50.5	41
Nuclear volume.....	Total	48.5 $\pm$ 3.6	89.5 $\pm$ 7.0	71.5 $\pm$ 7.9
	"Nerve"	45	78.5	63.5
(Nuclear volume) <sup>2</sup> .....	Total	155,000 $\pm$ 14,000	74,000 $\pm$ 11,000	66,000 $\pm$ 8,000
	"Nerve"	155,000	71,000	65,500
(Nuclear volume) <sup>3/2</sup> .....	Total	2,400 $\pm$ 200	2,300 $\pm$ 230	2,100 $\pm$ 240
	"Nerve"	2,300	2,200	2,000

\* These represent the standard errors of the means. For the average function per cell for each tissue these were calculated as described in table 1. For the total surface, volume, etc. per milligram the standard errors were calculated by the formula for a product. For example, if the average nuclear volume per cell in a given tissue is  $V \pm \sigma V$ , and the number of cells per milligram is  $N \pm \sigma N$ , the average nuclear volume per milligram is

$$NV \pm \sigma NV, \quad \sigma NV = \sqrt{V^2(\sigma N)^2 + N^2(\sigma V)^2}$$

volumes corresponding to nuclei in each dimension group were summed in numbers proportional to (by the factor, total nuclei per cubic millimeter over 1000) the nuclei found in that dimension area in the plot (fig. 5) of the desired

brain region. The average nuclear volume was then obtained by dividing this total volume per cubic millimeter by the cells per cubic millimeter. Such total and average nuclear functions were calculated, for each of the brain regions intensively studied, on the basis of all cells (1000 for each region) measured in ten experiments. Similar calculations were also made for the neural elements only, after excluding blood and capillary cells. The results are given in table 5.

DISCUSSION. Bok (1936) has published exhaustive quantitative studies of the neurone population of several areas of the human cortex, supplemented by some on primate cord (1934). He reports many striking relationships between nuclear parameters and other cell dimensions and distributions. Thus, for various cytoarchitectonic regions, with few large or many small neurones, total nuclear volume squared ( $\Sigma V^2$ , the sum of  $V^2$  of all individual nuclei) is constant. Strictly proportional to  $V^2$  is the cytoplasmic mass of each neurone (the perikaryon volume, equal to the volume of cell body after excluding nucleus and processes); and also the "territory" governed by each neurone—the volume occupied by its dendrites and by the terminals of other cells connecting with these—which is 35 times the perikaryon volume. The total length of all nerve processes is constant per unit brain volume, and so proportional to  $\Sigma V^2$ ; and the dendrite length of each neurone varies as its own  $V^2$ , so that total dendrite length is also constant per unit brain volume. Thus, all brain regions have a constant proportion of perikarya, dendrites, and incoming fibers, but each is characterized by the size of the individual cell units—and all these quantities vary directly as  $V^2$  or  $\Sigma V^2$ .

The surface area of the perikaryon is also a function of its nuclear volume, but in this case directly proportional to  $V$ ; whereas the total volume of each cell body (nucleus and cytoplasm) is proportional to  $V^{3/2}$ . Thus, per unit volume of grey matter, cytoplasmic mass, dendrite length, and total nerve fiber length vary as  $\Sigma V^2$ ; perikaryon surface varies as  $\Sigma V$ ; and protoplasmic volume as  $\Sigma V^{3/2}$ .

This study covers three widely differing brain regions in the frog, each extending from pia to ventricle and so presumably including the full "territories" of the contained neurones. An inverse relation between size and number of cells in these regions is at once apparent, but  $\Sigma V^2$  is not a constant. Either, therefore, such attributes as cytoplasmic mass and dendrite length are not proportional to  $V^2$  in frog neurones or their total values are not constant per brain mass.  $\Sigma V$ , and presumably perikaryon surface, is also not constant from one brain region to another.  $\Sigma V^{3/2}$  is, however, fairly constant in these frog brain regions and so, perhaps, is the volume of cell protoplasm.

Interestingly, the fraction of brain volume occupied by cell bodies decreases—and that available for synaptic field increases—from frog to man. Thus, Donaldson (1911) estimated cell bodies to occupy 2 per cent of the human brain and 3 to 5 per cent of the rat brain, and Bok finds almost 3 per cent of human cortex occupied by cell cytoplasm; while we find 6 to 8 per cent occupied by nuclei alone in the frog. Truszkowski's finding (1928), that the ratio of purine to total nitrogen is 50 per cent higher in frogs than in mammals, suggests a similarly greater nuclear mass in frog tissues as a whole.

Before correlating the respiration data with the cytological findings, some consideration of the oxygen consumption of non-neural cells is necessary. Measurements of the respiration of frog blood gave an average  $QO_2$  of 36; and of urostyle bone marrow (as somewhat representative of endothelial elements) a  $QO_2$  of 74 for a preparation with  $1.6 \times 10^5$  cells per milligram. The average brain sample contained 0.03 mgm. of blood and  $10^4$  endothelial cells per milligram. These would thus account, respectively, for 1 and 5 cm.  $O_2$  per hour per gram of brain—entirely negligible amounts in comparison with the measured  $QO_2$  values of 400 to 500. It is also to be noted that, since respiration and cell measurements are available on the same brain bits, weighing and other sampling errors will cancel rather than cumulate when oxygen consumption is calculated in terms of cells rather than of total mass.

TABLE 6

*Respiration as a function of the parameters of the cell nuclei of each brain region*

FUNCTION	CELLS	HIPPOCAMPUS	CEREBELLUM	ANT. OLF. NUC.
$QO_2/\text{cu.mm.}O_2/\text{gm.}/\text{hr.}$ .....		$475 \pm 7$	$400 \pm 8$	$390 \pm 7$
$O_2$ uptake per cell (cu.mm. per hr. $\times 10^{-6}$ ) .....	Total	9.1	1.7	3.1
	"Nerve"	13.5	2.5	3.9
$QO_2$ per unit surface (cu.mm. $O_2/\mu^2/\text{hr.} \times 10^{-9}$ ) .....	Total	$22.3 \pm 1.8^*$	$6.4 \pm 0.5$	$8.3 \pm 0.9$
	"Nerve"	26.8	7.9	9.3
$QO_2$ per unit volume (cu.mm. $O_2/\mu^3/\text{hr.} \times 10^{-9}$ ) .....	Total	$9.7 \pm 0.7$	$4.5 \pm 0.4$	$5.4 \pm 0.6$
	"Nerve"	10.5	5.1	5.7
$QO_2$ per unit (volume) <sup>2</sup> (cu.mm. $O_2/(\mu^3)^2/\text{hr.} \times 10^{-12}$ ) .....	Total	$3.1 \pm 0.3$	$5.4 \pm 0.8$	$5.8 \pm 0.7$
	"Nerve"	3.1	5.6	6.0
$QO_2$ per unit (volume) <sup>3</sup> (cu.mm. $O_2/(\mu^3)^3/\text{hr.} \times 10^{-12}$ ) .....	Total	$200 \pm 17$	$175 \pm 18$	$185 \pm 22$
	"Nerve"	205	195	195

\* The standard error, of the respiration per particular tissue dimension, is calculated on the basis of the statistics of the component variables by a formula for the standard error of ratios (see J. B. Scarborough, *Numerical Mathematical Analysis*, The Johns Hopkins Press, Baltimore, 1930).

$$\sigma \left( \frac{\bar{x}}{\bar{y}\bar{z}} \right) = \frac{\bar{x}}{\bar{y}\bar{z}} \sqrt{\left( \frac{\sigma\bar{x}}{\bar{x}} \right)^2 + \left( \frac{\sigma\bar{y}}{\bar{y}} \right)^2 + \left( \frac{\sigma\bar{z}}{\bar{z}} \right)^2}$$

Where  $\bar{x}$ ,  $\bar{y}$  and  $\bar{z}$  are the means respectively for respiration, cell number, and nuclear volume. We are indebted to Miss Eleanor Gish for assistance with all of the statistical calculations in this paper.

Table 6 gives the respiration as a function of various cell parameters for each brain region. It is obvious at a glance that, while respiration per milligram is but little greater in H than in AON or C, respiration per cell is 3 to 5 times as great—H having few large cells—while respiration per mass of cell protoplasm ( $\Sigma V^{3/2}$ ) is essentially alike in all. Further study shows that, assuming Bok's relations, the respirations of different brain masses are alike only when expressed as a function of the total nerve cell protoplasm contained in each, and not when correlated with the summated: volumes of nuclei or of cytoplasm alone, surfaces of nuclei or of cell bodies, lengths or surfaces of cell processes. Thus these findings, somewhat disappointingly, point to the whole protoplasmic mass rather than to surface membranes as determining the magnitude of the resting metabolism and the energy requirement of neurones.

## SUMMARY

1. Bits of frog brain tissue, about 1 mgm., were dissected from widely different structural regions: anterior olfactory nucleus (AON), primordium pallii (PP), hippocampus (H), ventral hypothalamus (VT), and cerebellum (C).

2. Respiration of these bits, as of nerve, was determined in a capillary respirometer; and the tissue was then macerated and the nuclei of nerve cells (and glia, capillary endothelium, and red blood cells) counted and measured. The method of cell evaluation is intrinsically valid to 5 per cent.

3.  $QO_2$  values, up to 700, are higher than most of those previously reported for frog and average about 40 per cent of those for mammalian brain. In order of descending  $QO_2$ , the series is C, H, AON, (PP), VT at first; with C falling off faster than other regions.

4. Nerve cells per milligram vary from 35,000 large neurones in H to 160,000 small ones in C, with a characteristic size population in each region. The total cell volume is roughly the same for all regions.

5. These data make it possible to express oxygen consumption as a function of the total neurones and, assuming the morphologic relations described by Bok, of the total neurite surface, perikaryon surface, process surface, nuclear surface, nuclear volume, perikaryon volume and total protoplasm volume. All these functions are analyzed statistically for valid correlations.

6. The respiration of various brain regions is constantly related to the mass of protoplasm, but not to the surface area, of the neural elements present.

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## OXIDASES, PRESSOR AMINES AND HYPERTENSION<sup>1</sup>

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According to Schroeder (1), high blood pressure can be treated with tyrosinase. The available enzyme preparations were found to be effective only when injected in large quantities, frequently resulting in toxic manifestations (1), typical for foreign proteins. Therefore, tyrosinase therapy of hypertension would be greatly improved if the dosage could be reduced considerably. It is conceivable that compounds could be found which facilitate or accelerate in vitro the enzymatic destruction of typical phenolic pressor amines, and which accelerate the action of tyrosinase in the hypertensive animal. The use of such a compound in conjunction with tyrosinase will probably permit the administration of smaller doses of the enzyme, thus reducing the possibility of anaphylactic reactions. Therefore, we determined the influence of various substances on the aerobic oxidation of adrenaline, a typical pressor amine, by tyrosinase. Moreover, since dopa is considered to be the precursor of the pressor amine arising in the kidney of hypertensive animals, the effect of substances found to alter the oxidative degradation of adrenaline by tyrosinase was investigated in dopa-kidney tissue systems.

**EXPERIMENTAL.** *Experiments with adrenaline-tyrosinase.* The in vitro enzymatic experimental procedure consisted in permitting the enzyme tyrosinase<sup>2</sup> to act at pH 7.1 in a medium composed of 2.5 ml. of a saline solution, 0.5 ml. of a 1:1000 dilution of adrenaline, and 2.0 ml. of a phosphate buffer solution. The test compounds were usually added in 1 mgm. amounts to this reaction mixture. After agitation for exactly twenty minutes at incubator temperature, a 2.0 ml. aliquot was immediately administered intravenously to cats or dogs and the effect on blood pressure observed. Under these experimental conditions, 2.0 ml. of a control solution, i.e., one which had been incubated in the absence of a test substance, was found to give an average pressor rise of 60 mm. of mercury. This response is the same as that obtained from the intravenous injection of 0.1 ml. of 1:10,000 adrenaline. With catechol as the test compound added in amounts varying from 0.001 to 1.0 mgm., no change in blood pressure was ob-

<sup>1</sup> Presented in part at Atlantic City, N. J. on September 11, 1941 (102nd Meet. Am. Chem. Soc., Div. Biol. Chem.).

<sup>2</sup> One-tenth milliliter of Stock Solution no. 2, prepared in the laboratory of Prof. J. M. Nelson of Columbia University, and containing approximately 1500 catechol-hydroquinone units (2) and 200 p-cresol units (3) per ml. measured at 25°C.

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served, indicating a complete destruction of adrenaline during the incubation period. When p-aminobenzoic acid in 1 mgm. quantities was the test substance, a rise of 120 mm., i.e., 60 mm. more than the control solution, was observed. This increase is the same as that obtained from the intravenous injection of 0.5 ml. of 1:10,000 adrenaline. Therefore, p-aminobenzoic acid seems to cause an inhibition of the destruction of adrenaline by tyrosinase. Apparently, calcium pantothenate and other B-complex vitamins likewise at the 1 mgm. level had no influence, the blood pressure rise being the same as that obtained when the control solution was injected.

TABLE 1  
*Catalysis of adrenaline oxidation by tyrosinase*

Column I	Column II	Column III
SUBSTANCES WITH AN INHIBITORY EFFECT*	SUBSTANCES WITH NO EFFECT†	SUBSTANCES WITH AN ACCELERATING EFFECT*
x‡ mgm.	x‡ mgm.	x‡ mgm.
<b>A</b>		<b>A</b>
Thiosalicylic Acid..... 0.01	Benzocaine..... 1.0	Catechol..... 0.01
p-Phenylenediamine..... 0.1	Calcium Pantothenate..... 1.0	o-Cresol..... 1.0
Aniline..... 1.0	Ferric Chloride..... 1.0	Aseptoform..... 1.0
Benzoic Acid..... 1.0	Hydroquinone..... 1.0	p-Hydroxybenzoic Acid..... 1.0
Orcinol..... 1.0	Inositol..... 1.0	Phenol..... 1.0
p-Aminobenzoic Acid..... 1.0	Nicotinic Acid..... 1.0	o-Aminophenol..... 0.1
m-Aminobenzoic Acid..... 1.0	Novocaine..... 1.0	
o-Aminobenzoic Acid..... 1.0	Pyridoxine HCl..... 1.0	
Pyrogallol..... 1.0	Sulfanilamide..... 1.0	
	Thiamine HCl..... 1.0	
<b>B</b>		<b>B</b>
Ascorbic Acid..... 1.0		p-Hydroxyphenylacetic Acid... 1.0
p-Nitrobenzoic Acid..... 1.0		Dopa..... 1.0
Ephedrine..... 1.0		

\* In order of decreasing action.

† In alphabetical order.

‡ x is the amount of test substance added to the tyrosinase-adrenaline mixture which was incubated at 37°C. for 20 minutes before being intravenously injected into a dog or cat. The mixture incubated without the test substance gave a 60 mm. blood pressure rise. This increase is the same as that obtained from 0.1 ml. of 1:10,000 adrenaline. Mixtures incubated with the substances listed in column IA gave a 120 mm. rise (same as 0.5 ml. of 1:10,000 adrenaline), with those of column IB, 110 mm. (same as 0.4 ml. of 1:10,000 adrenaline), with those of column II, 60 mm., with those of column IIIA, no change in blood pressure, and with those of column IIIB, a slight increase (same as 0.05 ml. of 1:10,000 adrenaline).

The various substances tested are classified in table 1 according to their influence on the destruction of adrenaline by tyrosinase. Column 1 shows the compounds which had an inhibitory influence on adrenaline destruction, as evidenced by a rise of blood pressure above that obtained with the control solution. In column 2 are the substances which were found to be inactive, and column 3 lists the compounds with an accelerating effect, as seen from the fact that only a small, if any, pressor response was observed.

*Experiments with dopa-kidney tissue.* As pointed out in the introduction, the various test substances were further studied in order to determine whether they play a rôle in the formation of pressor amines by the kidney. For this purpose, 2 grams of minced kidney tissue from dog or cat was incubated at pH 7.1 for

about 18 hours in 30 ml. of a phosphate buffer solution containing 40 to 60 mgm. of dopa. The test compounds were added in concentrations of 1 to 10 mgm. per 30 ml. of the mixture to be incubated. The incubated product was centrifuged, and 2 to 10 ml. of the supernatant liquid was injected intravenously into a cat or a dog, and its effect on blood pressure recorded.

Under these experimental conditions it was found that substances increasing (table 1, column 3) the oxidation rate of adrenaline by tyrosinase, or decreasing (table 1, column 1) this rate, also influence in the same manner the production of pressor substances by kidney tissue acting on dopa. In other words, the experiments showed that there is a parallelism between the results in the dopa-kidney system and those of the adrenaline-tyrosinase studies already described.

*Experiments with perinephritic-hypertensive dogs.* Compounds which promote in vitro the destruction of adrenaline by tyrosinase (table 1, column 3) also facilitate the action of the latter in reducing blood pressure. It was found, namely, that 15,000 catechol-hydroquinone units of tyrosinase are required intramuscularly to reduce the blood pressure of the experimentally produced hypertensive dog from 230 to 150 mm., and that only 500 units of the same tyrosinase preparation are necessary when catechol is first administered at a level calculated to bring the concentration to  $10\gamma$  per ml. of blood. Since dopa may act as catechol in catalysing the destruction of adrenaline, the experiments were repeated using dopa in place of catechol. An aqueous suspension of 100 mgm. of dopa was injected intramuscularly immediately preceding the intramuscular administration of 700 units of tyrosinase. The blood pressure of 230 mm. did not fall, as it did in the catechol experiments, but was further elevated. This result is seemingly not in accord with the data of table 1 showing a similarity of catechol and dopa in their effect on tyrosinase activity. However, it is in harmony with the results obtained in the dopa-kidney tissue studies already described. Apparently, dopa may be decarboxylated to produce a pressor amine which is hydroxytyramine, according to Bing (4).

**DISCUSSION.** Ortho substituted phenols (e.g., catechol, cresol, aminophenol) and compounds such as dopa and p-hydroxybenzoic acid facilitate the enzymatic destruction of adrenaline. Catechol is particularly potent, in fact, even ten times more effective than when allowed to influence the non-enzymatic oxidation of adrenaline.

The three isomeric aminobenzoic acids and several structurally related substances inhibit the enzymatic destruction of adrenaline. Aminobenzoic acids were shown by Woolfe (5) not to protect adrenaline against non-enzymatic in vitro oxidation. These compounds do not destroy tyrosinase, nor are they themselves oxidised in preference. Therefore, their observed activity is probably due to enzyme blockage. An additional attribute may thus be added to the list of properties of p-aminobenzoic acid, i.e., inhibition of the enzymatic oxidation of adrenaline, already confirmed by the Warburg technique, the results of which showed a direct correlation between oxygen consumption (6) and those obtained in the present communication by pharmacological testing.

The study of adrenaline oxidation by tyrosinase brought out the fact that there exists a definite structural relationship among the catalysing benzene

derivatives investigated. Compounds with an amino group, carboxyl radical, or both, in ortho, meta, or para position on the ring, have an inhibitory action as, e.g., aniline, benzoic acid, and the isomeric aminobenzoic acids. The introduction of a hydroxyl radical into the inhibiting molecules of aniline and benzoic acid produces an aminophenol and a hydroxybenzoic acid, respectively, both of which were found to accelerate tyrosinase action, although by different processes. Structural relationship plays no rôle when protection is afforded to adrenaline by substances which do not block the enzyme as, e.g., ascorbic acid which is inherently oxidised with ease. Thiosalicylic acid is definitely non-specific in its action, since it inhibits the oxidation by tyrosinase irrespective of whether the substrate is p-cresol, l-tyrosine, l-dopa, adrenaline, or catechol-ascorbic acid (7).

No marked specificity among the test compounds studied was seen in the adrenaline-tyrosinase systems, benzoic acid, aniline, and the three isomeric aminobenzoic acids being effective in protecting adrenaline. This result is particularly noteworthy in view of the observations on the anti-sulfanilamide action of para-aminobenzoic acid and local anesthetics, such as benzocaine and novocaine derived therefrom, and the ineffectiveness as anti-sulfonamides of ortho and meta aminobenzoic acids, benzoic acid, and p-nitrobenzoic acid (8, 9).

Dopa occupies a unique position in the systems studied. It forms a pressor amine when incubated with minced kidney tissue, and yet it is capable of catalysing the oxidative destruction of adrenaline. Therefore, the behavior of a given compound at a given concentration in a tyrosinase system need not indicate its ultimate effect in the hypertensive animal. A molecule which inhibits tyrosinase and catalyses decarboxylase reactions will be materially different in its *in vivo* action from one which inhibits both tyrosinase and decarboxylase.

#### SUMMARY

1. The oxidative destruction of adrenaline, a typical pressor amine, is promoted in a tyrosinase-adrenaline system by ortho-substituted phenols and inhibited by aromatic amines and aminobenzoic acids.

2. The destruction of pressor bodies formed during incubation of dopa-kidney tissue systems is altered by the test compounds in the same manner as in tyrosinase-adrenaline systems.

3. The effectiveness of tyrosinase in reducing blood pressure of the perinephritic-hypertensive dog is enhanced by the simultaneous administration of catechol.

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# STUDIES OF THE METABOLISM OF BOVINE EPIDIDYMAL SPERMATOZOA<sup>1</sup>

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The metabolism of mammalian spermatozoa has been studied previously. Attempts to identify the source of energy for motility and the development of methods for the assay of the fertilizing capacity of semen have been in the foreground of such investigations. The studies were based partly on chemical determinations of metabolic products, partly on manometric measurements of the gaseous exchange.

The present experiments were performed to confirm and extend observations made by several authors on the metabolism of bovine spermatozoa (1-4) which should serve as a basis for more extensive investigations of the effect of various reagents on the activity of these cells, particularly in relation to the enzyme systems present. Some initial studies of this nature have been completed (5, 6). Aspects of respiration and glycolysis of epididymal spermatozoa have been studied and factors responsible for variations in oxygen consumption have been analyzed. Results obtained with a few samples of seminal spermatozoa are included.

**MATERIALS AND METHODS.** *Spermatozoa.* Bovine testicles were obtained daily from the abattoir 1 to 2 hours after the death of the animals. Suspensions of spermatozoa were prepared in Ringer solution under conditions minimizing bacterial contamination as described elsewhere (7). Spermatozoa obtained from 6 to 10 epididymides were usually pooled. These heavy suspensions were diluted with Ringer solution so as to yield suitable concentrations for the tests. In a few instances the spermatozoa were washed once or thrice by slow centrifugation and subsequent resuspension in Ringer solution.

For a number of experiments bull semen was used.<sup>2</sup> A few of the samples were about 3 hours old; others had been kept at refrigerator temperature for 18 hours before use. The spermatozoa were sedimented in the centrifuge at low speed and resuspended in Ringer solution.

In the early work the spermatozoa were counted in a blood counting chamber after suitable dilution of the suspensions. In many instances the counting was

<sup>1</sup> This work has been aided by a grant from the National Committee on Maternal Health, Inc.

<sup>2</sup> Obtained through the courtesy of the Veterinary School of the University of Pennsylvania.

checked by determining the dry weight of the cells washed twice in distilled water and dried at 100°C for 18 hours. The average weight of 100 million bovine spermatozoa was found to be 2.4 mgm. More recently the counting has been replaced by measuring the turbidity of the spermatozoal suspensions by means of the Klett-Summerson photoelectric colorimeter. The number was estimated from a standard turbidity curve for suspensions of spermatozoa which were counted and diluted so as to yield concentrations of approximately  $10^7$ ,  $2 \times 10^7$ , and so forth, up to  $10 \times 10^7$  cells per ml. The results obtained by counting and turbidimetry agreed within 10 per cent.

*Media.* Ringer solution containing 0.85 per cent NaCl, 0.2 per cent KCl, and 0.2 per cent  $\text{CaCl}_2$  was used as basic medium. Usually glucose was added in a concentration of 100 to 200 mgm. per cent. In many cases M/15 phosphate buffers of different pH were added to the spermatozoal suspensions, ratios of 1:5 or 1:2 being used. For pH higher than 8.0 M/15 borate buffer was employed in the same ratios. For the determination of glycolysis sodium bicarbonate was added to the Ringer solution to a final concentration of 0.025 M.

*Methods.* The oxygen consumption of the spermatozoal suspensions was measured by means of the direct method of Warburg under air at 37°C. (8). Vessels with a central cup and one side-arm (volume 16 to 17 ml.) were employed. The  $\text{CO}_2$  was absorbed by 0.3 ml. of 20 per cent KOH, which was placed in the central cup together with a piece of filter paper. The rim of the cup was covered with a thin layer of paraffin to prevent the alkali from creeping over into the spermatozoal suspensions. Two milliliters of the suspensions were always employed. Usually 2 vessels were used for each determination. The manometers were shaken at 110 oscillations per minute.

For the evaluation of the oxygen consumption a modification of the Warburg quotient

$$\left( Q_{O_2} = \frac{\text{cmm. O}_2}{1 \text{ mgm. dry weight/hour}} \right)$$

was used (1b):

$$Z_{O_2} = \frac{\text{cmm. O}_2}{100 \text{ million spermatozoa/hour}}$$

This value may be converted into the more frequently used  $Q_{O_2}$  by dividing the  $Z_{O_2}$  values by 2.4, the dry weight of 100 million spermatozoa.

The respiratory quotient and aerobic glycolysis were determined in Dixon-Keilin vessels (8) under a mixture of oxygen and 5 per cent  $\text{CO}_2$ . The vessels were connected with Warburg manometers filled with Clerici solution. The calculations were made according to the second method of Dickens and Šimer (8).

In other cases the aerobic glycolysis was measured by the direct method of Warburg likewise under the  $\text{O}_2$ - $\text{CO}_2$  mixture. As the respiratory quotient of bull spermatozoa in the presence of glucose is 1.0 (5), this method can be substituted for the technique mentioned above. Comparison of the results obtained by both methods with the same spermatozoal suspensions showed negligible discrepancies.

The anaerobic glycolysis was determined by the direct method of Warburg in an atmosphere of nitrogen with 5 per cent  $\text{CO}_2$ . The glycolytic values are expressed as:

$$Z_{\text{G}}^{\text{O}_2} = \frac{\text{cmm. CO}_2 \text{ liberated by aerobic lactic acid formation}}{100 \text{ million spermatozoa/hour}}$$

and

$$Z_{\text{G}}^{\text{N}_2} = \frac{\text{cmm. CO}_2 \text{ liberated by anaerobic lactic acid formation}}{100 \text{ million spermatozoa/hour}}.$$

These values were calculated from the measurements for the first 30 minutes of an experiment.

*Motility.* The motility of the suspensions was checked at the end of each experiment and is described when pertinent to discussion of the results.

**EXPERIMENTAL.** *Oxygen consumption of bovine spermatozoa.* In confirmation of others (1, 2) bovine spermatozoa obtained from the epididymis were found to consume oxygen. Cells prepared as described and suspended in glucose-free Ringer solution showed a relatively high initial rate of oxygen consumption but it soon decreased (*A*, fig. 1) until it reached a rate ( $Z_{\text{O}_2}$ ) of 3 to 4 cmm. oxygen for 100 million cells per hour. If glucose was added to the medium the oxygen uptake increased markedly and remained at the higher level (*B*, fig. 1), sometimes at a constant rate for 3 to 4 hours, when the experiments were terminated.  $Z_{\text{O}_2}$ -values as high as 25.0 were encountered under these conditions. Spermatozoa washed once or thrice and suspended in glucose-free Ringer solution did not show the initial high oxygen consumption of the unwashed cells (*C*, fig. 1.) and the  $Z_{\text{O}_2}$  for the first hour was already low and not higher than 5.0. This indicated that some substrate was transferred from the epididymis to the suspensions responsible for the higher respiration of unwashed cells in the first hour of the experiment but removed by washing of the cells. When glucose was added to washed spermatozoa the respiration increased to a level similar to that of the unwashed cells under the same conditions (*D*, fig. 1).

The motility apparently did not depend on the addition of glucose to the medium. At the end of the experiments washed spermatozoa in glucose-free medium exhibited motility comparable to cells suspended in Ringer glucose. Very dense suspensions of spermatozoa without added glucose showed motility even after 48 hours at room temperature.

It was found that as little as 1 mgm. per cent glucose when added to washed spermatozoa produced a short lived increase of respiration, the duration of which depended, of course, on the number of spermatozoa present. When 10 mgm. per cent of glucose were added to the medium, the respiration remained at the higher level for the length of the experiment, and further increase in the glucose concentration up to 300 mgm. per cent did not alter the  $Z_{\text{O}_2}$ -values. At no time in these experiments with epididymal spermatozoa did the addition of glucose cause a reduction in the oxygen consumed as described recently by Lardy and Phillips (4) for seminal spermatozoa.

The oxygen consumption of epididymal spermatozoa in the presence of glucose varied in the different experiments from 8.6 to 25.5 cmm. per 100 million cells. This variation called for further analysis and several factors were found to influence the oxygen uptake.

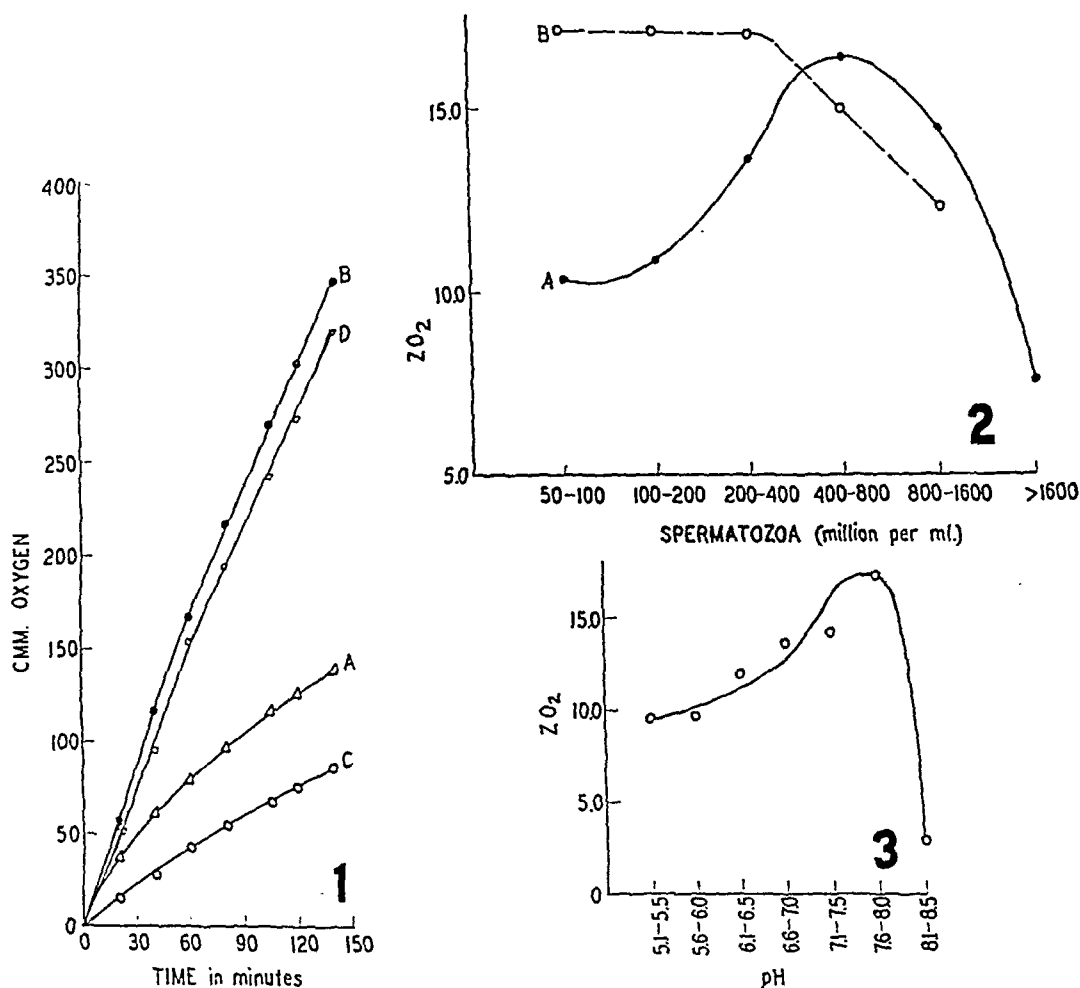


Fig. 1. Oxygen consumption of unwashed and washed epididymal spermatozoa ( $330 \times 10^6$  cells/ml) in Ringer solution with or without glucose. A = Unwashed spermatozoa in glucose-free Ringer. B = Unwashed spermatozoa in Ringer-glucose. C = Three times washed spermatozoa in glucose-free Ringer. D = Three times washed spermatozoa in Ringer-glucose.

Fig. 2. Influence of concentration of spermatozoa on oxygen consumption and the effect of epididymal secretion. A = Spermatozoa in Ringer-glucose. B = Spermatozoa in Ringer-glucose and epididymal secretion.

Fig. 3. Influence of pH on oxygen consumption of epididymal spermatozoa.

1. *Differences in individual samples.* Spermatozoal suspensions prepared from individual epididymides showed appreciable differences in respiration, as shown in table 1. The motility of the cells and the pH of the suspensions at the end of the experiments did not differ sufficiently to furnish an explanation for these variations.



2. *Influence of the degree of maturity.* Since spermatozoa undergo a process of maturation during their passage through the epididymis (9), attempts were made to obtain spermatozoa in various stages of development, i.e., from the testicle, the caput and the cauda of the epididymis, and from the vas deferens. However, it was found impossible to recover sufficient numbers of cells from some of the locations, and the spermatozoal suspensions were not free of blood and tissue cells. On the other hand, several samples of seminal spermatozoa,

TABLE 1

*Oxygen consumption of bovine spermatozoa derived from individual epididymides, suspended in Ringer-glucose*

SAMPLE NO.	NUMBER OF SPERMATOZOA PER ML.	Z <sub>O<sub>2</sub></sub>			
		1st hour	2nd hour	3rd hour	Average
1	147 × 10 <sup>6</sup>	-10.7	-10.7	-10.8	-13.8
2	201 × 10 <sup>6</sup>	-20.6	-14.1	-14.4	
3	166 × 10 <sup>6</sup>	-13.3	-10.3	-10.4	
4	149 × 10 <sup>6</sup>	-12.5	-9.9	-10.8	
5	213 × 10 <sup>6</sup>	-22.6	-16.1	-16.2	
1-5	180 × 10 <sup>6</sup>	-15.2	-12.0	-12.1	-13.1

TABLE 2

*Oxygen consumption of bovine seminal spermatozoa in Ringer-glucose*

SAMPLE	NUMBER OF SPERMATOZOA PER ML.	AGE	MOTILITY	Z <sub>O<sub>2</sub></sub>		
				1st hour	2nd hour	3rd hour
J	215 × 10 <sup>6</sup>	18 hrs.	+	-9.7	-6.9	-4.4
K	210 × 10 <sup>6</sup>	18 hrs.	+	-10.3	-5.6	-5.3
R	220 × 10 <sup>6</sup>	18 hrs.	-	0.0	0.0	0.0
10	500 × 10 <sup>6</sup>	3 hrs.	++	-6.9	-4.9	-3.5
9	450 × 10 <sup>6</sup>	3 hrs.	+	-7.6	-6.0	
epid. sp*	240 × 10 <sup>6</sup>	18 hrs.	++	-16.4	-15.7	-11.3
epid. sp†	345 × 10 <sup>6</sup>	18 hrs.	±	-7.0	-7.0	-7.2

\* Whole epididymis kept in refrigerator.

† Spermatozoal suspension kept in refrigerator.

++ = very good motility; + = moderate motility; ± = majority immobile; - = no motility.

studied about 3 hours after emission showed an oxygen consumption decidedly lower than cells derived from the epididymis. Other samples, tested 18 to 20 hours after emission, until which time they had been kept in the refrigerator, consumed oxygen at a similar rate. Some of the data are recorded in table 2. One sample (R) which contained only immobile spermatozoa did not use oxygen. Suspensions of epididymal spermatozoa which were kept in the refrigerator for 18 hours showed similarly low values while storage of the whole epididymis did not affect the respiration of the spermatozoa subsequently prepared.

3. *Influence of the concentration of spermatozoa on the oxygen uptake.* It was

observed that higher  $Z_{O_2}$ -values frequently corresponded with a high spermatozoal count in the suspension. Experiments using various dilutions of the same suspension confirmed the observation. From 14 experiments an average curve has been computed (fig. 2, A) by plotting  $Z_{O_2}$ -values against the number of spermatozoa per milliliter. It is obvious from this figure that maximal respiration took place when the spermatozoal suspensions contained 400 to 800 million cells per milliliter. With higher concentration the oxygen uptake decreased. This fact could be due either to the effect of crowding with a resulting decrease of spermatozoal activity (cf. 10), or, particularly in the highest concentrations, to insufficient diffusion of oxygen into the suspensions. For the decrease in oxygen uptake encountered in dilute spermatozoal suspensions two possible explanations were considered: a, a factor in the epididymal secretion may stimulate the respiration if present in sufficient concentration, but is rendered ineffective by dilution although the actual amount of this substance per

TABLE 3

*Influence of epididymal secretion on oxygen consumption of epididymal spermatozoa in Ringer-glucose ( $Z_{O_2}$ )*

NUMBER OF SPERMATOOA PER ML.	CONTROL	EPIDIDYMAL SECRETION 10 MCM. PER ML.	PER CENT INCREASE
210 $\times 10^6$	-9.8	-15.3	56
220 $\times 10^6$	-8.4	-15.7	87
425 $\times 10^6$	-10.3	-12.3	19
150 $\times 10^6$	-9.4	-16.6	77
220 $\times 10^6$	-13.7	-19.1	39
205 $\times 10^6$	-10.0	-15.5	55
220 $\times 10^6$	-9.8	-9.5*	

\* Seminal fluid added instead of epididymal secretion.

spermatozoön would remain constant; b, products of spermatozoal metabolism may enhance the oxygen uptake in more concentrated suspensions, while they may not reach an effective level in dilute preparations.

In order to test these points the respiration of spermatozoal suspensions was studied in Ringer-glucose with or without the addition of "epididymal secretion". This material was obtained by collecting large amounts of spermatozoa in distilled water and separating the cells by centrifugation at 2000 r.p.m. for 30 minutes. The opalescent supernate was saved and dried from the frozen state. The reddish-brown brittle material was added to the spermatozoal suspensions by weight so as to yield a 1 per cent solution. Table 3 shows that this material stimulated appreciably the oxygen uptake of spermatozoa. The increase in respiration varied in some 20 experiments from 19 to 87 per cent over the controls, the average being 40 per cent. The epididymal secretion alone did not absorb oxygen or, if so, only minimal amounts, and the results of the experiments were corrected accordingly. The motility of the spermatozoa was not influenced by the agent.

As little as 2 mgm. of the material per milliliter gave the stimulating effect. Freshly obtained epididymal secretion gave a stronger effect than the dried material obtained from spermatozoal suspensions collected over several days. This indicated its epididymal origin rather than its derivation from spermatozoal metabolism or autolysis. The factor was found to be heat stable, withstanding 30 minutes at 56°C. or even 10 minutes at 100°C. without loss of activity. It was not dialysable through cellophane, i.e., the residue stimulated the respiration to approximately the same degree as the original material and the concentrated dialysate was without effect. The addition of seminal fluid to epididymal spermatozoa did not influence the oxygen uptake.

The increase in oxygen consumption induced by the addition of the epididymal secretion clearly depended on the concentration of spermatozoa in that the higher increases were observed in dilute suspensions, while in preparations containing more than 500 million cells per milliliter no stimulation was noticed or even a slight depression took place. Curve *B* of figure 2 represents the average of 4 such experiments. It shows that the drop in respiration encountered in dilute suspensions of the control spermatozoa can be prevented completely by the addition of the epididymal secretion.

4. *Influence of pH.* Epididymal spermatozoa suspended in Ringer solution showed a pH of 6.0 or slightly less. This agreed with the pH of the cauda of the epididymis which was found by Redenz (9) to be approximately 5.8. The pH of bull semen has been found to be 6.5 to 7.5 (11). When epididymal spermatozoa were suspended in Ringer-glucose solution buffered at various pH the respiration showed an optimum between pH 7.5 and 8.0 both in phosphate and borate buffers, and so did the motility. Above this value the oxygen uptake dropped rapidly while below the optimal pH the respiration fell more gradually until a pH of 5.0 was reached, the lowest pH studied with the phosphate buffer. These relationships are demonstrated in figure 3, which represents an average curve drawn from eight different experiments covering this range of pH.

5. *Influence of various media.* It is reasonable to assume that media of various composition will influence the oxygen uptake of bovine spermatozoa. However, this point has not been studied adequately and only experiments comparing the respiration in Ringer-glucose and in Ringer-bicarbonate-glucose have been made. The oxygen uptake was markedly increased in the latter medium and  $Z_{O_2}$ -values of over 30 were encountered. The motility also seemed more active in this medium than in the bicarbonate-free preparations.

*Aerobic and anaerobic glycolysis.* Manometric data concerning the aerobic and anaerobic glycolysis of bovine spermatozoa are available through the studies of Redenz (1), who used dialyzed serum as medium. In the present study the spermatozoa were suspended in Ringer-bicarbonate solution to which 200 mgm. per cent of glucose had been added. Table 4 shows some of the results which agree well with the observations of Redenz. The values for aerobic and anaerobic glycolysis varied to a certain degree in different samples but less than the oxygen consumption. The concentration of spermatozoa per milliliter did not play a rôle comparable to that in the respiration experiments nor did the epididymal secretion influence the lactic acid formation.

Since only very few samples of bovine seminal spermatozoa were available for the determination of glycolysis, a comparison between these and epididymal cells in regard to glycolysis does not seem justified, although in the few tests performed the rate of anaerobic glycolysis appeared somewhat smaller in the seminal spermatozoa. MacLeod (12) observed that human seminal spermatozoa showed an aerobic glycolysis amounting to 80 per cent of the anaerobic one. In the present experiments with bovine epididymal spermatozoa the aerobic glycolysis was found to be 40 to 60 per cent of the anaerobic values. Two samples of seminal spermatozoa showed an aerobic glycolysis of 60 and 73 per cent of the anaerobic lactic acid formation.

DISCUSSION. In accordance with Redenz (1) it was found that the oxygen consumption of bovine epididymal spermatozoa varied over a wide range. In analyzing this phenomenon it was observed that spermatozoa derived from individual epididymides showed different oxygen needs. Spermatozoa undergo a process of maturation while passing through the epididymis (9). On frequent emission the cells become more and more immature, while on the other hand

TABLE 4  
*Aerobic and anaerobic glycolysis of bovine spermatozoa*

SOURCE OF SPERMATOZOA	NUMBER OF SPERMATOZOA PER ML.	$Z_{\text{G}}^{\text{O}_2}$	$Z_{\text{G}}^{\text{N}_2}$	$Z_{\text{G}}^{\text{O}_2}$ AS PER CENT OF $Z_{\text{G}}^{\text{N}_2}$
Epididymis	$910 \times 10^6$	56.7	95.4	59.4
Epididymis	$470 \times 10^6$	37.5	95.7	39.2
Epididymis	$340 \times 10^6$	37.7	73.0	51.6
Epididymis	$115 \times 10^6$	38.0	84.0	45.3
Epididymis	$130 \times 10^6$	48.6	81.2	60.0
Epididymis	$157 \times 10^6$	38.2	76.3	50.0
Semen	$500 \times 10^6$	44.0	60.0	73.3
Semen	$450 \times 10^6$	44.5	73.4	60.6

spermatozoa stored for a long period in the epididymis lose gradually their fertilizing capacity (cf. 13). These physiological changes may account for some of the individual differences found in this study in that spermatozoa of varying degrees of maturity may have different metabolic requirements. Although the various developmental stages could not be tested, results obtained with seminal and epididymal spermatozoa showed that the oxygen uptake was so much lower in the former that there is no doubt that respiration is less in seminal cells. This difference has to be studied further. Possible interaction of the secretions of the accessory glands may be excluded since the addition of seminal fluid to epididymal cells did not alter their oxygen consumption. Lardy and Phillips (4) found that the oxygen consumption of seminal cells is lower in the presence of glucose than in glucose-free medium, a fact we can confirm for seminal spermatozoa but not for epididymal cells.

Optimal oxygen consumption took place in suspensions containing 400 to 800 million cells per milliliter. The drop in respiration encountered in excessively concentrated suspensions may be ascribed to insufficient diffusion of oxygen into the medium or may partly be due to a crowding effect (cf. 10). The decreased

oxygen uptake in dilute suspensions of spermatozoa could be overcome by the addition of epididymal secretion to the medium. The agent present was found to be heat stable and not dialysable. Seminal fluid did not influence respiration.

Finally the pH may alter the oxygen uptake, optimal respiration taking place at pH 7.5 to 8.0. By controlling the various factors mentioned it has been found possible to obtain more uniform results and the variation in oxygen consumption observed under these conditions was not greater than encountered in other mammalian cells and tissues.

In agreement with others (3, 4, 12 and others), a high aerobic glycolysis was found in addition to the anaerobic glycolysis. There is no doubt that the glycolysis furnishes the major part of energy for spermatozoal activity in the presence of glucose. However, respiration and other processes may substitute under certain conditions (cf. 14).

#### SUMMARY

Variations in the oxygen uptake of bovine epididymal spermatozoa, suspended in Ringer solution, were shown to be due *a*, to differences in the individual samples; *b*, to the concentration of spermatozoa, optimal respiration taking place in suspensions containing 400 to 800 million cells per ml.; *c*, to the pH of the suspensions, highest oxygen consumption occurring at pH 7.5 to 8.0; *d*, to possible differences in the degree of maturity of the cells. Seminal spermatozoa showed a decidedly lower oxygen consumption than epididymal cells.

An analysis of the phenomenon of optimal concentration showed that while in excessively concentrated suspensions mechanical hindrance of the spermatozoa or insufficient diffusion of oxygen into the medium could account for the lower oxygen uptake, the decreased respiration in dilute suspensions of spermatozoa was completely prevented by the addition of epididymal secretion to the medium. The factor present in this material is heat stable and not dialysable.

The high aerobic glycolysis of epididymal cells, amounting to 40 to 60 per cent of the anaerobic reaction, was confirmed. The concentration of the spermatozoa did not influence the amount of glycolysis and epididymal secretion did not alter the results.

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# THE USE OF DOUBLE WORK PERIODS IN THE STUDY OF FATIGUE AND THE INFLUENCE OF CAFFEINE ON RECOVERY<sup>1</sup>

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A review of the literature reveals that a "single work period" performed one or more times per week is the method most commonly employed for the study of the effect of various factors on voluntary motor fatigue (1-10). The published observations show that training, "staleness" and other known and unknown factors cause a variation in the output of work from period to period. The factors of training, "staleness," motivation or the "will to do," are factors difficult to control. Our work was undertaken to ascertain whether double work periods, in which the subject works to exhaustion, then rests for a brief period and then again works to exhaustion, might be more advantageous for the study of fatigue than the "single work period." It was believed that the per cent recovery might be more constant than the output of work during a "single work period." Certain observations reported by a number of investigators indicated that such might be the case (11-17). In fact, one investigator (17) has studied the effect of massage, etc., on the per cent of recovery.

This paper presents the results obtained regarding whether the per cent of recovery and other items of data provided by double work periods is more constant than the output of work during a single work period. Results on the effect of caffeine on the per cent of recovery and the output of work during a second work period after a ten minute period of rest are also presented.

Caffeine was chosen as the drug to use in these tests because it is generally recognized to be a drug which increases the work output in voluntary muscular fatigue in ergometric tests (18, 19) as usually performed. However, the drug has not been administered intravenously in man to ascertain its effect on recovery from fatigue or rather its effect on voluntary muscular effort in the presence of fatigue. The nearest approach was made by Graf (14, loc. cit. Bøje) who administered caffeine and cola nuts after partially fatiguing his subjects.

**METHODS.** Six medical students were used. They were provided with board and room in a hospital near the laboratory. Their food intake was under control; their diet was adequate in all known components, a certain variation in the selection of food items being permitted. The subjects were chosen on the basis of their willingness to coöperate, and not on the basis of muscular development, the work of the tests being their only source of physical exercise. The

<sup>1</sup> Aided by the Abbott Fund of Northwestern University.

double work periods occurred on Monday, Wednesday and Friday afternoons at the same time between 2 and 4 o'clock.

The subjects worked on the bicycle ergometer described by Hellebrandt and Kelso (20). After trying various rates, loads and rest periods on a relatively large number of subjects, a rate of work of 1235 kg. m. per minute with a pedalling rate of 54 r.p.m. was chosen for the tests. Three to five preliminary workouts on the bicycle were allowed for the development of a skill pattern.

*The subjects worked to complete fatigue, rested ten minutes, and then worked to complete fatigue again.* The ten minute rest period was chosen because some subjects if given longer periods of rest manifested complete recovery. Indications that the subjects had gone the limit each time were profuse perspiration, uncontrollable muscular twitching, spasm and contracture of the quadriceps, leg

TABLE 1

*Comparison of the variability of the first period, second period, and total work output as well as that of per cent recovery*

SUBJECT	PERIOD OF TRAINING	NUMBER OF DOUBLE WORK PERIODS	AVERAGES WITH STANDARD DEVIATION				COEFFICIENT OF VARIATION (PER CENT)			
			1st period work output	2nd period work output	Total work output	Per cent recovery	1st period work output	2nd period work output	Total work output	Per cent recovery
	<i>months</i>		<i>Kg. m.</i>	<i>Kg. m.</i>	<i>Kg. m.</i>					
A	2 -3	19	13,951 $\pm$ 1913	6356 $\pm$ 677	20,275 $\pm$ 2529	46 $\pm$ 5.5	13.72	10.65	12.48	11.95
B	1.5-3.75	21	6,573 $\pm$ 813	4491 $\pm$ 1447	11,142 $\pm$ 1197	68 $\pm$ 5.67	12.23	32.21	10.76	8.34
C	2.5-3.75	19	10,378 $\pm$ 1859	6125 $\pm$ 744	16,510 $\pm$ 2213	60 $\pm$ 9.11	17.93	12.14	13.41	15.20
D	2 -3.25	15	10,029 $\pm$ 803	5595 $\pm$ 417	15,540 $\pm$ 902	56 $\pm$ 5.70	8.02	7.45	6.21	10.17
Average of A, B, C, D. ....						57 $\pm$ 6.5	13.22	15.59	10.71	11.41
E	0 -1.5	17	2,592 $\pm$ 426	2107 $\pm$ 276	4,700 $\pm$ 686	81 $\pm$ 5.55	16.42	13.08	14.59	6.80
F	0 -1.5	12	2,408 $\pm$ 359	1946 $\pm$ 280	4,355 $\pm$ 630	81 $\pm$ 3.41	14.92	14.38	14.45	4.20
Average of E and F .....							15.67	13.73	14.52	5.50
Average of all .....						$\pm$ 5.82	13.87	14.99	11.98	9.44

buckling, leg pains and slight nausea. The end-point of fatigue was the point at which they could no longer hold the needle on the dial at the rate of 54 r.p.m.

RESULTS. A summary of a portion of the data on the work output of the six subjects is shown in table 1. It is to be noted that in the case of the first four subjects no data are given for the first 1.5 to 2.5 months. This is because these subjects were used during that period to survey various factors that might influence the duration of the first and second work periods, such as the load, pedalling rate, etc., factors that we desired to study but which do not pertain to the subject of this paper. Hence, the data shown for the first four subjects represent those obtained after a brief period of training. The data on subjects E and F, however, represent the initial period of training. It is to be noted that the average standard deviation of the *per cent recovery* for each subject is relatively small and that the average standard deviation of the group is only 5.82

per cent. The coefficient of variation (21) of the per cent of recovery is less than that for the first work period in all cases, except subject D, and less than that for the second work period in three of the six subjects. In the case of subjects E and F, who started untrained with the standard rate of work, the data represent their process of early training. Both have shown substantial increases in work output with training. In the case of subject E the work output during the first period was 2.4 times more variable than his per cent of recovery; in the case of subject F, 3.5 times more variable.

*Comment.* In the first place the double work period, as used, provides four items of data which may be studied statistically instead of the one item provided by the single work period. Secondly, it is evident that in five of the six subjects

TABLE 2

SUBJECT	PERIOD OF TRAINING	NUMBER OF DOUBLE WORK PERIODS	AVERAGES WITH STANDARD DEVIATION OF THE DISTRIBUTION				COEFFICIENT OF VARIATION (PER CENT)			
			1st period work output	2nd period work output	Total work output	Per cent recovery	1st period work output	2nd period work output	Total work output	Per cent recovery
A. With intravenous placebo of NaCl solution										
	months		Kg. m.	Kg. m.	Kg. m.					
A	4 -6	11	9,948 $\pm$ 1,822	5880 $\pm$ 358	15,830 $\pm$ 1972	60.7 $\pm$ 9.52	18.30	9.36	12.46	15.68
B	4 -6	10	8,560 $\pm$ 249	4722 $\pm$ 214	11,280 $\pm$ 427	71.9 $\pm$ 3.15	3.80	4.53	3.78	4.38
E	1.5 -3.25	10	3,836 $\pm$ 289	2777 $\pm$ 157	6,614 $\pm$ 426	72.7 $\pm$ 3.29	7.54	5.66	6.44	4.52
F	1.25-3	10	3,993 $\pm$ 480	2594 $\pm$ 256	6,522 $\pm$ 717	65.3 $\pm$ 4.45	15.27	9.88	10.99	6.81
Averages.....						67.49 $\pm$ 5.85	11.23	7.36	8.42	7.85
B. With intravenous caffeine-sodium benzoate 0.5 gram										
A	4 -6	11	10,043 $\pm$ 1313	9006 $\pm$ 1167	20,891 $\pm$ 2231	91.5 $\pm$ 18.64	13.06	12.96	10.68	20.37
B	4 -6	10	6,481 $\pm$ 294	5559 $\pm$ 285	12,039 $\pm$ 481	86 $\pm$ 4.90	4.54	5.13	4.00	5.70
E	1.5 -3.25	10	3,606 $\pm$ 380	2836 $\pm$ 112	6,442 $\pm$ 483	79.2 $\pm$ 5.74	10.54	3.95	7.50	7.25
F	1.25-3	10	3,641 $\pm$ 467	2595 $\pm$ 348	6,236 $\pm$ 789	71.4 $\pm$ 4.63	12.83	13.41	12.65	6.48
Averages.....						82.2 $\pm$ 10.6	10.24	8.86	8.76	9.95

\* One gram of caffeine sodium benzoate was required to produce the same effects in subjects E and F as were produced in subjects A and B with the half-gram dose.

the per cent recovery showed less variation than the output of work during the first work period. In the exceptional case, subject D, the total work output as well as that during the first or second work periods is less variable than the per cent of recovery. In the case of subjects E and F, it may be concluded that any test factor or drug, introduced at random during the period of training, which increases the per cent of recovery two or more times the standard deviation above the mean of the placebo tests, has a favorable effect on recovery. Thus, the effect of drugs and other quickly acting substances on the fatigue mechanisms can be assayed during the training period, and the results would be as reliable as results obtained after a period of training. Referring to table 2A, it will be noted that the coefficient of variation of the per cent recovery of the same subjects (E and F) is significantly no more variable during the first portion of the



period of training than later. The same is only relatively true of subject A, but subject B has become more consistent with further training.

*Comparison of the variation of the work outputs of the first work periods of our subjects with those of others.* Doctor Hellebrandt provided us with data on six control subjects who started training on the bicycle ergometer under standard conditions, as did our subjects E and F. She used only a "single work period." The average coefficient of variation during the first 15 work periods (first 5 omitted to allow for development of a skill pattern as was done in our tests) in her subjects was found to be 14.05 per cent, and that of our two comparable subjects, E and F, during the first work period was 15.67 per cent, which is remarkably close.

Four of her subjects continued the tests for two months and the other two for six weeks longer. For the tests performed after the initial training period the average coefficient of variation of the four subjects stopping at two months was 9.17 per cent, and for the two stopping at 3.5 months was 5.0 per cent. The average coefficient of variation of the per cent of recovery in subjects E and F during the first six weeks of training was 5.5 per cent. This indicates that per cent recovery, as a criterion of fatigue, is in some subjects as constant during the first six weeks of training with subjects working only 3 days a week as is the single daily workout after a period of two months of training in some subjects. Referring to table 2A, which summarizes our data on four subjects during a period of from 1.5 to 6 months, it is seen that the coefficient of variation during the first work period in the different subjects varies from 3.8 to 18.3 per cent, giving an average of 11.23 per cent. Two of our subjects, B and E, fell within the range of the two of Doctor Hellebrandt's subjects who worked for 3.5 months. But it should be noted that in 3 of our 4 subjects (table 2A) the per cent of recovery was less variable than the work output of the first work period.

Further evidence of the constancy of per cent recovery was obtained by interpreting Kent's (13) data statistically. He gives 60 consecutive per cent recoveries on one subject. Thirty were taken in the morning and 30 in the evening. The average morning recovery was  $90 \pm 9.51$  per cent, giving a coefficient of variation of 10.56 per cent. The average evening recovery was  $88.5 \pm 9.08$  per cent, giving a coefficient of variation of 10.26 per cent. The initial and second work outputs were not given. Considering the fact that Kent's data were collected using the finger ergograph with a 5 minute rest period, it is remarkable that the average coefficient of variation of 10.41 per cent, calculated from his data, so closely approximates our average coefficient of variation of 9.44 per cent.

In regard to the use of the *finger ergograph*, we initially started to use the extensor ergograph, modified after Maison (22), but were dissatisfied with the variation observed from day to day. In our subject the average 1st period work output for 33 tests was  $11.7 \pm 4.89$  kgm. giving a coefficient of variation of 43.78 per cent. Doctor Maison kindly permitted us to analyze the data he had obtained on himself; the coefficient of variation of the first twenty-five work periods was 47.51 per cent. Even using the extensor ergograph with a 1 minute rest period, we found the per cent recovery in our one subject was  $40 \pm 11.6$  per

cent, giving a coefficient of variation of 29 per cent. This is supported by interpreting Lamb's data (25) statistically. He used an arm ergograph and fatigued the right arm in 28 subjects and the left in 25. The subjects were untrained and each served for one test. The arm was worked to fatigue, rested 10 minutes, and worked to fatigue again. The average coefficient of variation for the 1st work period (both arms) was 41.25 per cent; that for the per cent recovery was 31.01 per cent. This indicates that *even in untrained individuals per cent recovery is a more constant physiological character than is the initial work output.*

*Individual differences.* The individual differences in the work output and per cent of recovery during the earlier and later portions of the training or experimental period should be noted in tables 1 and 2. In the case of subject A, the per cent recovery increased after three months; however, the total work and especially the work output during the first period decreased. Subject B has manifested little change in recovery and work output after 3 months; he is a very consistent subject. Subjects C and D discontinued the experiment after 3.5 months. Subjects E and F manifested a decrease in per cent recovery after 1.5 months, but the total work output and especially that of the first period increased.

The averaged data (tables 1 and 2) as well as the data of the individual tests show that the per cent of recovery is inversely related to the work output of the first period, as might be anticipated, even though the former is less variable; the correlation coefficient is 0.95. As the subject trains and becomes capable of a greater output during the first period, the per cent recovery becomes less. This indicates that a given subject recovers from fatigue more rapidly when he works to "exhaustion" during the early period of training, when the output of work is relatively small, than he does later when the output of work is greater. As training progresses, the load becomes relatively lighter for the muscles and the factors concerned in fatigue may accumulate less acutely; this permits the accumulation of a greater debt, which requires a longer recuperative period. As training progressed, the subjects reported that pain in the muscles became less significant as a cause of cessation of work. Another point of interest is that with additional training the output of work during the second period became less variable (tables 1 and 2) and on the average (table 2) was slightly less variable than the per cent of recovery. With training the coefficient of variation of the per cent of recovery may increase or decrease; it increased in subjects A and F, and decreased in subjects B and E (tables 1 and 2).

*The Effect of Caffeine. Methods.* Four subjects were used. Two (A and B) had been trained for three months, and two (E and F) for about 1.5 months. Immediately after the first work period, the subjects were injected at random intravenously with a placebo (2 cc. NaCl solution) and 0.5 gram of caffeine-sodium benzoate (2 cc.) or 0.25 gram caffeine. Ten minutes after the end of the first work period, the subjects worked to exhaustion again. The two solutions were colorless. Coffee had not been taken by any of the subjects for several months, and was not taken during the experiment. Subject A weighed 155 lbs.; B, 155 lbs.; E, 140 lbs.; and F, 180 lbs.

*Results.* The summarized data are shown in tables 2A and 2B. The average

*per cent recovery* for the four subjects was  $67.49 \pm 5.85$  with the placebo and  $82.2 \pm 10.6$  with the caffeine. The effect of caffeine was definite; the critical ratio of the difference is 7.7. The critical ratio for the difference between the placebo and caffeine tests in the case of subject A is 4.4, and subject B, 4.8; for the other two subjects, it is 2.7. Referring to the *work output during the second period*, the critical ratio for the difference between the placebo and caffeine tests for subject A is 7.6; for subject B, 6.6; for subjects E and F there is no difference. Referring to the *total work output*, the critical ratio for the difference between the placebo and caffeine tests for subject A is 5.1; for subject B, 3.3; and for subjects E and F there is no significant difference.

*Comment.* In the case of subjects A and B, the critical ratios (greater than 3) of the differences between the placebo and caffeine tests are significant for per cent recovery, for work output during the second period or the period after caffeine, and also for the total work output for both periods. The caffeine, thus, had a definite effect on subjects A and B. Although the critical ratio for the difference between the placebo and caffeine tests relative to the per cent of recovery comes close to being significant, the lack of a difference between the output of work during the second period indicates that the difference in per cent recovery is probably due to chance, in the case of subjects E and F.

The reports of the influence of caffeine on the feelings of the different subjects correlate with the objective results just cited.

Subjectively A and B reported that when they received the caffeine, though they did not know what it was, they felt less fatigued during the rest period and that they believed the onset of fatigue and muscular pain during the second work period was delayed. Subject E was unable to tell whether he received caffeine or a placebo and F had a bitter taste after the caffeine, but had no other sensations. Apparently the dose of caffeine used was not sufficient to influence subjects E and F. Hence, the increased output of work by subjects A and B is best ascribed to the elevation in mood and the decrease in the sensation of fatigue produced by caffeine. However, it should not be forgotten that subjects A and B were doing more work than subjects E and F. In subjects E and F a one gram dose of the drug was required to produce objective and subjective effects similar to those obtained by a half gram dose in subjects A and B.

Graphic records of *hand tremor* showed that caffeine increased the tremor slightly; the records were made directly after the end of the second work period. The caffeine had no effect on *breath holding* tests made 10 minutes after the end of the second work period.

#### SUMMARY AND CONCLUSIONS

Six subjects under controlled conditions of housing and diet were studied to ascertain whether a "double work period" might be more advantageous for the study of fatigue than the "single work period." A bicycle ergometer and a rather heavy load was used, 1235 kg. m. per minute with a pedalling rate of 54 r.p.m. Three times each week the subjects were worked to exhaustion, and after ten minutes of rest were worked to exhaustion again. In three of four subjects,

A, B and C, who had been previously trained for a period of from 1.5 to 3.75 months, the per cent of recovery was found to be less variable than the work output of the first or second periods. The total work output for the two periods (subjects A, B, C, and D) varied to about the same extent as the per cent of recovery. In two subjects, E and F, who had not been previously trained, the per cent of recovery was less variable during 1.5 months of training than any other item of the data; the total work output for the two periods ranked second. Further, training of these two subjects for a total period of 3 months showed that the per cent of recovery continued to be the least variable. Further, training of subjects A and B for a total period of 6 months showed the work output during the second period to be the least variable. *In general, as the length of the training period increases the work output during the second period, the total work output for both periods and the per cent recovery become less variable and approximately equal in variability; the work output during the first period remains the most variable of the four items of data.* The double work periods in three of four of our subjects even after considerable training yielded three items of data less variable than the single or first work period (table 2).

Another advantage of the double work periods is that it permits a drug or rapidly acting substance to be assayed during the training period, so that each subject may serve as his own control. In addition, it permits a study of the effect of rapidly acting substances on recovery.

It was found, as might be anticipated, that the per cent recovery under our experimental conditions is inversely related to the work output of the first period; the correlation coefficient was approximately 0.95.

Caffeine-sodium benzoate (0.5 gram) injected intravenously at the end of the first work period increased the per cent recovery or the output of work during the second period in the two subjects that were subjectively affected by the drug. It had no significant effect in the two subjects who were not subjectively affected; in these subjects a one gram dose of the drug was required to produce objective and subjective effects.

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# THE CIRCULATORY RESPONSE OF THE UNANESTHETIZED DOG TO SMALL PHYSIOLOGICAL QUANTITIES OF ADRENALIN

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There is general agreement that the injection of small quantities of adrenalin produces marked changes in arterial blood pressure and readjustments in blood distribution (1, 2 and others). Several factors may participate in determining the direction and magnitude of this response (3). For example, the low blood pressure which follows pithing may be elevated by the injection of adrenalin in amounts which prior to pithing elicit a depressor response (4). A similar reversal of the adrenalin depressor response occurs when anesthesia deepens (5). Minimal effective doses of this drug are said to reduce the arterial blood pressure in the etherized dog (6) and in the decerebrate cat (7). Such findings suggest that adrenalin depressor responses should be readily elicited in normal, unanesthetized animals. Dragstedt and his associates (8), however, encountered only pressor responses to adrenalin injections in unanesthetized dogs. They postulated in addition that the minimal effective dose of adrenalin for these animals ranges from 0.2 to 0.4  $\mu\text{g.}/\text{kgm.}/\text{min.}$  In the present study an attempt has been made to investigate the circulatory effects of small physiological doses of adrenalin in the unanesthetized dog with the aid of an optical manometer system.

**METHODS.** Seven dogs which were accustomed to the technical procedure served as the experimental animals. In all experiments the dogs were unanesthetized and had been deprived of food for at least sixteen hours. The animals rested quietly for thirty to forty-five minutes before any measurements of the blood pressure were made. The marked phasic sinus arrhythmia, normally present in quiet dogs, was occasionally reduced or eliminated by the onset of panting induced by high environmental temperature and humidity. It is worthy of mention, however, that the hemodynamic changes in such instances were identical both in direction and magnitude with those observed during quiet breathing.

Systolic and diastolic pressures were recorded from the femoral arteries by means of a modified Gregg hypodermic optical manometer system (9). Hypodermic puncture of the jugular vein was also carried out for the administration of adrenalin. To eliminate any disturbances associated with the act of puncturing arteries and veins, 30 to 60 seconds were allowed to elapse before actual registration of blood pressures was begun.

When arterial pressures are determined by this technique, the slightest blood

clotting within the lumen of the needle may produce bizarre pulse contours and, consequently, unreliable ordinate pressure values. Systemic anti-coagulants cannot be employed because the procedure requires repeated arterial punctures. The adrenalin response has therefore been studied for short intervals only following single injections of the drug. The blood pressure was recorded upon rapidly moving photosensitive paper. This procedure permitted careful study of the individual pulse curves by means of which coagulation within the system was detected and the faulty records discarded.

As a precautionary measure against deterioration of the adrenalin, fresh, concentrated stock solutions (1:1000) were diluted to the desired strength immediately prior to administration. Distilled water and physiological saline served as alternate diluents. The arbitrarily selected quantities of adrenalin (0.1, 0.2 and 0.3  $\mu\text{g./kgm.}$ ) were prepared in volumes which never exceeded 3 ml. of fluid and were administered via the jugular veins. External compression of the veins was carefully avoided. The rate of injection was varied from 0.019 to 0.1  $\mu\text{g./kgm./sec.}$

**RESULTS.** A special procedure for record analysis was adopted in order to discount the large fluctuations in blood pressure determinations which are primarily respiratory in origin. After the systolic and diastolic values for every individual pulse had been determined, each record was arbitrarily divided into six-second pre- and post-injection intervals, commencing with the onset of injection. An average of the pressures for each interval was obtained and the respective heart rates counted. The average blood pressures from interval to interval in the control periods were very constant. In 28 observations of control blood pressures, the systolic and diastolic pressures averaged 190 and 90 mm. Hg respectively. The latter compare favorably with normal femoral pressure values similarly procured by Hamilton (10) in 215 unanesthetized dogs. An average heart rate of 97 beats per minute was obtained from all control records. If, however, the records registered on panting dogs are excluded, the latter value is lowered to 87 beats per minute. Blood pressure levels were not measurably affected by the onset of panting.

The necessity of insuring adequate separation of the individual pulse curves entailed photographic registration of exceptionally long records. Since even the inclusion of typical segments of the latter was found impractical, the experimental observations are presented in tabular and graphic form. The direction and magnitude of the maximal circulatory responses from individual experiments are entered in table 1. Associated with the latter are *a*, the dose of adrenalin employed, *b*, the rate of injection, and *c*, the total volume of fluid administered. In these studies it was impossible to detect any significant correlation between the rate of injection and either the direction or magnitude of the adrenalin depressor response. A similar lack of correlation has been more recently observed in experiments in which large physiological pressor doses were administered at rates as great as 0.1  $\mu\text{g./kgm./sec.}$  (11). Control injections of the diluents in volumes equivalent to the maximal quantities required for adrenalin administration altered neither the heart rate nor the blood pressure.

There is evidence, however, that the adrenalin concentration per unit of animal weight determines the direction of the circulatory response. In 13 of 14 tests, for instance, arterial pressures were definitely lowered following injection of 0.1  $\mu\text{g.}/\text{kgm.}$  quantities of this drug. As the strength of adrenalin was

TABLE 1

DOG NO.	EXP. NO.	DOSE	DOSE	MAX. CHANGE IN S.P.	MAX. CHANGE IN D.P.	MAX. CHANGE IN H.R.	ADRENALIN INJECTED	NATURE OF RESPONSE
		$\mu\text{g.}/\text{kgm.}$	$\mu\text{g.}/\text{kgm.}/$ <i>second</i>				<i>ml.</i>	
1	19	0.1	0.071	-28	-18	+25	0.78	Depressor
	20	0.1	0.071	-26	-11	+14	0.78	Depressor
	22	0.2	0.100	+4	+6	+5	1.76	
	21	0.3	0.100	+11	+13	-9	2.34	
	25	0.3	0.050	-22	-9	+42	2.43	Depressor
2	13	0.1	0.019	-26	-15	+20	0.86	Depressor
	15	0.1	0.037	-23	-22	+44	0.86	Depressor
	16	0.2	0.044	+26	+15	-25	1.70	
	17	0.3	0.050	+55	+29	-40	2.60	
	20	0.3	0.070	+25	+17	-22	2.65	
3	17	0.1	0.009	-25	-11	+30	0.60	Depressor
	20	0.1	0.009	-11	-14	+26	0.60	Depressor
	21	0.2	0.040	-34	-10	+45	1.16	Depressor
4	12	0.1	0.044	+30	+2	+15	0.66	
	13	0.1	0.026	-16	-5	+13	0.66	Depressor
	30	0.1	0.080	-22	-2	+38	0.66	Depressor
	14	0.2	0.080	-21	-2	+52	1.32	Depressor
	15	0.3	0.070	-20	-5	+43	1.98	Depressor
5	19	0.1	0.034	-34	-26	+34	1.18	Depressor
	20	0.1	0.040	-32	-17	+36	1.18	Depressor
	21	0.2	0.040	-50	-22	+27	2.36	Depressor
6	1	0.1	0.039	-27	-8	+40	0.94	Depressor
	5	0.1	0.027	-11	-3	+30	0.99	Depressor
	11	0.2	0.083	+18	+11	+40	1.98	
	2	0.3	0.071	-26	-9	+36	2.82	Depressor
7	7	0.1	0.040	-12	-4	+14	1.13	Depressor
	12	0.3	0.088	+17	+10	-15	3.15	
	5	0.3	0.100	-16	-15	+33	3.42	Depressor

increased, pressor elevations were also observed and the number of depressor responses obtained became less frequent. For example, the depressor response was encountered in only 66 and 63 per cent of the experiments in which 0.2 and 0.3  $\mu\text{g.}/\text{kgm.}$  doses were respectively introduced. With 0.5  $\mu\text{g.}/\text{kgm.}$  dosages, 18 of 20 trials revealed pressor reactions. Excluding the 0.1  $\mu\text{gm.}$  doses, all



other quantities of adrenalin were occasionally observed to elicit dual responses, characterized by an initial depression and a secondary rise in the blood pressure.

It is difficult to detect the factor or factors which govern the magnitude of this depressor response, particularly since each of the specific doses included in table 1 has, at one time or another, produced both great and small reductions in arterial pressure. The largest reductions in systolic and diastolic pressures encountered were respectively 50 and 26 mm. Hg and the smallest 11 and 2 mm. Hg. The average systolic and diastolic reductions were 25 and 11 mm. Hg.

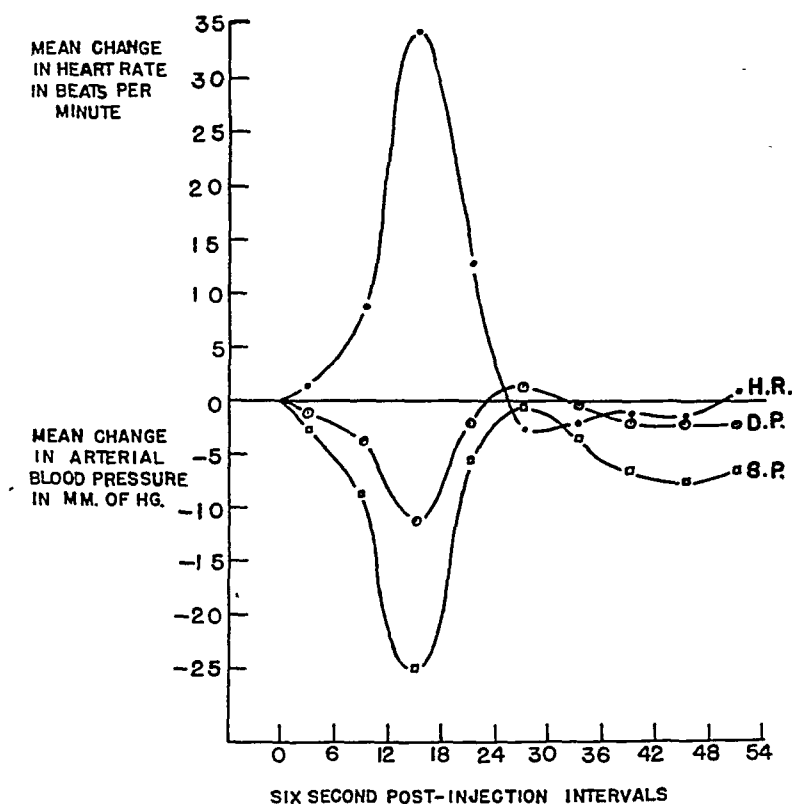


Fig. 1. Mean duration-response curves compiled from 28 experiments in which a depressor response was obtained with 0.1, 0.2 and 0.3  $\mu\text{g.}/\text{kgm.}$  doses of adrenalin. H.R., heart rate; D.P., diastolic pressure; S.P., systolic pressure.

Since the respiratory fluctuations have been previously discounted in the calculations, these changes are significant.

Inasmuch as the *directional* changes in heart rate and blood pressure were so strikingly uniform during the respective post-injection intervals of each experiment, it seems permissible to average the results and present them as mean duration-response curves (fig. 1). The latter represent typical time relations of the directional alterations seen in practically all adrenalin depressor reactions. It is apparent that changes in heart rate and blood pressure began within 8 to 9 seconds after the onset of injection and that within 12 to 18 seconds, the maximal effects were attained. With almost equal suddenness, the curves were reversed

in direction, and initial pre-injection values were obtained within the 24 to 30 second interval. For some reason, not yet apparent, the systolic pressure frequently underwent a secondary depression of lesser magnitude than the original fall. When this occurred, normal systolic values were again reached before or during the 60 to 66 second interval.

Two features of this adrenalin response merit special attention. When a dose of adrenalin, which originally elicited a depressor phenomenon, was repeated at twenty-minute intervals, the initial response was always reproduced. It appears, therefore, that the unanesthetized dog differs from the decerebrate cat in which similarly repeated injections were totally ineffective (7). The other interesting feature is the unanticipated predominant reduction of the systolic blood pressure.

**DISCUSSION.** The experimental findings confirm Dragstedt's contention that doses of adrenalin under  $0.2 \mu\text{g./kgm.}$  are incapable of initiating any significant elevation of arterial pressure. They are not, however, in accord with the belief that these concentrations are unable to affect the blood pressure level. Nor do they agree with the concept that doses which vary from  $0.2$  to  $0.4 \mu\text{g./kgm.}$  are strictly minimal effective *pressor* doses. For example, a definite depressor effect was almost uniformly seen in response to  $0.1 \mu\text{g./kgm.}$  quantities and a fall in blood pressure was more common than an elevation when the so-called "minimal effective pressor doses" were employed.

Despite the differences in the technical procedures adopted by the two groups of investigators, the factors underlying the discrepancies in the results are not easily determined. The rate of injection affords no clue since no correlation between rate of injection and the blood pressure response was observed. Moreover, the rates employed by the two groups were essentially equivalent in many instances. Nor can these divergencies be attributed to the selection of the carotid artery by one investigator and the femoral by the other, since it is common knowledge that, in most instances, mean pressures in the two vessels agree. The initial effects of a single injection of adrenalin should be visible in the early effects of a continuous infusion. It is surprising, therefore, that Dragstedt and his co-workers failed to detect even an initial transient fall in arterial pressure with  $0.1$  and  $0.2 \mu\text{g.}$  quantities of adrenalin. The impression is gained from a careful examination of their published records that, at least in some instances, these reactions, which are less intense than most pressor effects, may have been partially masked by excessive respiratory undulations which were superimposed upon the non-critically damped pressure excursions as transmitted by a mercury manometer. A complete and satisfactory answer to this problem is still wanting.

The adrenalin depressor response is interesting because the dynamic mechanisms involved may be a part of the system of homeostatic buffering devices provided for bodily protection against sudden elevations of arterial blood pressure. The intimate regulation of epinephrine discharge from the adrenal medulla by the nervous system renders it not unlikely that, among other events, brief emotional disturbances may elevate circulating epinephrine to concentra-

tion levels which simulate those experimentally established in this investigation. In such circumstances, however, a reduced arterial blood pressure is not necessarily to be anticipated. Being less discrete, nature's excitatory agents may synchronously enlist other vasomotor mechanisms which are pressor in character. Consequently, the net effect of the interplay between the antagonistic mechanisms may mask any gross indications that the adrenalin depressor mechanisms are engaged at the time. Nevertheless, the dynamics of this event are worthy of further consideration.

The fall in blood pressure elicited by adrenalin has hitherto been investigated in anesthetized and decerebrate animals and has received a variety of circumstantial interpretations (12). The most widely accepted concept postulates a dilatation of smaller arteries, particularly those in skeletal muscles, and hence a reduced systemic peripheral resistance. It is understood, however, that a lessening of peripheral resistance *per se* favors a predominant reduction in diastolic pressure, providing aortic and arterial elasticity are not grossly abnormal. So far as the results obtained in this investigation are concerned, the greater diminution of the systolic pressure relegates the systemic peripheral resistance factor to a rôle of minor importance. Supplemental reasons for dismissing the latter as a cardinal participant are afforded *a*, by the brief latency between the injection and the initial circulatory response, and *b*, by the abrupt attainment of maximal effects within 12 to 18 seconds after the onset of injection.

Although positive evidence for the explanation of this reaction is unavailable from the data, further consideration reveals two hitherto neglected factors. If, for instance, the pulse pressure pattern of the adrenalin depressor effect were to be reproduced in an artificial circulation model, it would be necessary to increase the aortic capacity or to decrease the stroke output of the left ventricle.

Little is known about the behaviour of the aortic walls in the intact animal in response to adrenalin. Recently, Wiggers and Wégria (13), while eliciting an acute adrenalin hypertension in barbitalized dogs, detected an initial (probably passive) enlargement of aortic capacity. This was abruptly terminated as peak pressures were attained when a secondary diminution (probably active) set in. From this and other studies on perfused isolated sections of the aorta it is reasonable to postulate that depressor doses of adrenalin may momentarily increase the capacity of the aorta and its immediate branches in the normal unanesthetized animal.

Left ventricular stroke volume might be significantly impaired *a*, by reducing the systemic venous return; *b*, by depressing ventricular contractility, or *c*, by reducing the pulmonary venous outflow and hence the filling of the left ventricle. There is little justification for invoking the first two factors to explain this response. The third has definite possibilities. There is abundant evidence (14) in its favor though unfortunately it is derived from perfusion experiments on isolated lungs and hence must be employed with considerable reservation. Adrenalin, in quantities comparable to the depressor dose, appears to augment the pulmonary venous outflow, *if*, among other things, a constant perfusion

pressure is maintained. This effect has been attributed to a dilatation of pulmonary vessels. If adrenalin dilates pulmonary vessels when administered to the normal animal, a somewhat different series of dynamic events may be anticipated. For instance, a fall in the pulmonary arterial (perfusion) pressure will follow any significant reduction of the pulmonary resistance such as may be occasioned by dilatation of vessels. Hence, a temporary pooling of blood within this capacious reservoir must occur with a consequent reduction of pulmonary venous outflow. Accordingly the left ventricle is less adequately filled and the systolic discharge is reduced. The tachycardia which abbreviates the diastatic inflow phase of successive cardiac cycles may also accentuate this condition. The ensuing reduction in left ventricular systolic discharge is thus the most likely dynamic factor responsible for the greater diminution of systolic than of diastolic pressure in the adrenalin depressor reaction.

#### CONCLUSION AND SUMMARY

It appears that the unanesthetized dog will exhibit either an elevation or a depression of blood pressure in response to small intravenous injections of adrenalin, the direction depending upon the dose per unit of animal weight. The depressor reaction, which can be uniformly elicited by  $0.1 \mu\text{g./kgm.}$  quantities, is reproducible in the same dog at twenty-minute intervals. Shorter intervals were not investigated. Slightly stronger concentrations of this drug may elicit either pressor or depressor effects, the latter being slightly more prevalent. Although the factors which govern the direction of the response are not completely understood, it is suspected that the initial emotional status of the animal is of considerable importance. The rate of injection does not appear to influence the direction of the response.

Since the depressor response is characterized by a predominant reduction of systolic pressure, it becomes increasingly difficult to accept the doctrine that a diminution of systemic peripheral resistance is the precipitant mechanism. We are more inclined to postulate an initial dilatation of pulmonary vessels with a consequent reduction of pulmonary arterial resistance and thus a temporary pooling of blood within the lung vessels. Hence, a transient reduction of left ventricular filling will ensue which will be further accentuated by the simultaneous tachycardia.

It has also been suggested that adrenalin may augment the capacity of the aorta and its immediate large branches. This would also exert directional effects upon the pulse pressure pattern similar to those resulting from a reduced stroke volume of the left ventricle. These two mechanisms may even act synergically in bringing about the adrenalin depression of arterial blood pressure.

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# THE ANTERIOR PITUITARY IN THE CARBOHYDRATE METABOLISM OF THE EVISCERATED RAT<sup>1</sup>

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Evidence has been presented which indicates that there is an accelerated peripheral utilization of carbohydrate in the hypophysectomized rat and rabbit. Hypophysectomized rats lose their carbohydrate stores during fasting at a much greater rate than do normal rats. In glucose-fed rats a smaller percentage of the absorbed carbohydrate can be accounted for, four hours after feeding, in the hypophysectomized animals. In both the fed and the fasting states, the operated animals have R. Q.'s sufficiently higher than the normal to indicate that the increased carbohydrate disappearance could be due to increased oxidation (1, 2, 3). In fasted animals the apparent effect may be due to diminished glyconeogenesis in the absence of the hypophysis, but the rates of nitrogen excretion in normal and in operated rats do not differ enough to indicate that this factor is of critical importance. In the fed rats, it is unlikely that differences in rates of glyconeogenesis are a factor in producing the observed differences in carbohydrate recovery, since this would require a massive new formation of carbohydrate in the normal rats in the presence of a plethora of carbohydrate.

Greeley (4) has shown that the hypophysectomized rabbit requires the administration of large amounts of glucose to maintain normal blood sugar levels during fasting, and that this requirement, about 500 mgm. per kilo per hour, is not further increased by evisceration or hepatectomy. This requirement is considerably greater than that of the normal eviscerated rabbit which, according to Drury (5), is about 200 mgm. per kilo per hour. No attempt was made by Greeley to determine the fate of the injected glucose, but he concluded on other grounds that the extra glucose requirement was probably due to increased oxidation.

Since, as has been mentioned, previous work suggests that there is an increased peripheral oxidation of carbohydrate in the hypophysectomized rat, it seemed desirable to confirm the existence of this phenomenon in the eviscerated rat. Such a simplified preparation makes it possible not only to exclude the effects of different rates of glyconeogenesis but also to determine how far muscle glycogen deposition and lactic acid formation contribute to the differences in carbohydrate

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utilization. In the present work it is shown that in the hypophysectomized eviscerated rat there is a greater rate of fall of blood sugar than in the normal eviscerated rat, that a greater amount of glucose is required to maintain the blood sugar within normal limits, and that neither of these differences can be due to increases in muscle glycogen deposition or in lactic acid formation.

**METHODS.** Young male rats of the Sprague-Dawley strain, weighing from 180 to 220 grams, have been used in these experiments. The animals were not fasted, since it was desired that the hypophysectomized animals be in the best possible condition, and that the normal and hypophysectomized rats should have blood sugar levels in the same range at the beginning of the experiments.

Hypophysectomy was performed by the parapharyngeal approach 2 to 4 weeks before the evisceration experiments. The removal of the anterior lobe was complete in all cases, as judged by growth stasis and by atrophy of the testes. Adrenal-demedullation was performed always 2 weeks or more prior to hypophysectomy, in order to allow time for regeneration of the adrenal cortex. Growth rates of the rats were normal after adrenal-demedullation.

The chemical methods used were the following: for glucose, in blood filtrates and in tissue hydrolysates, the methods of Somogyi (6) or of Shaffer and Somogyi (7); for preparation of blood filtrates, Somogyi's copper tungstate reagents; for glycogen, a modification of the method of Good, Kramer, and Somogyi (8); for total carbohydrate, a modification of the procedure of West and Peterson (9); for lactic acid, the *p*-hydroxy-diphenyl color reagent, by the procedure of Barker and Summerson (10), with the Evelyn colorimeter. The latter method, with slight changes, has proven quite satisfactory in the determination and recovery of very small amounts of lactic acid.

Tail blood (0.2 ml.) and gastrocnemius muscles were used for most analyses.

Constant intravenous infusion was maintained when desired by use of a device designed and built by the Johnson Foundation, University of Pennsylvania. Special precautions were taken in the design and use of this apparatus to insure perfectly uniform infusion rates. In each of these experiments, a volume of 2 ml. per hour of fluid, consisting of 1 to 2.5 per cent glucose in 0.9 per cent sodium chloride, was infused into a femoral vein. The rate of delivery of glucose by the apparatus was determined by analysis in each experiment.

"Functional" evisceration was the procedure employed here; that is, the abdominal viscera, with the exception of the liver, were removed, and the blood vessels supplying the liver—the coeliac axis and the portal vein—were tied and cut. This operation was used, rather than absolute evisceration, because it can be performed very quickly, in 2 or 3 minutes, and without any hemorrhage or any signs of shock, even in hypophysectomized or adrenalectomized rats.

That the liver remaining in situ is effectively cut off from the blood stream is suggested by the correspondence of the survival periods and rates of fall of the blood sugar in these eviscerated rats with those in completely hepatectomized rats, determined by Selye and Dosne (11). Moreover, the lactic acid content of the asphyxiated livers of these animals may be as much as 200 mgm. per cent, with no corresponding increase in blood lactic acid. Finally, the changes in

total carbohydrate content of the livers *in situ* have been determined directly, by analyses of the livers in several experiments. Samples were taken immediately after the evisceration and again 2 hours later for determinations in duplicate of total carbohydrate, lactic acid, and dry weight. In six animals, with initial total liver carbohydrate of from 0.44 to 1.13 per cent, small and uniform changes occurred; the average loss of total carbohydrate was 0.062 per cent, and the lactic acid content increased 0.046 per cent. The net loss of 16 mgm. per cent corresponds to a loss of approximately 0.3 mgm. per 100 grams of rat per hour. Even these small losses disappeared entirely when the results were calculated upon a dry weight basis, since there was usually an imbibition of water by the asphyxiated livers. Since these data were obtained from animals not given glucose, in which the blood sugar was falling rapidly, opportunity for loss of carbohydrate from the liver was maximal. Therefore also in the glucose-infused animals, there was probably no contribution of the livers to the blood sugar.

The eviscerations were carried out under nembutal and very light ether anesthesia, and the animals were maintained under light nembutal anesthesia throughout the subsequent experimental periods. Rectal temperatures of all animals were maintained at 99° to 101°F. Emptying of the bladder occurred often, indicating active kidney function. No artificial ventilation was required, and the respiratory rate continued uniform throughout the course of each experiment.

**RESULTS AND DISCUSSION.** In the first experiments of this series, in which intact animals were eviscerated and the changes in muscle glycogen were determined, it was noticed that a marked loss of muscle glycogen occurred during the operations, an average value of 440 mgm. per cent glycogen being obtained immediately after the evisceration. A further large and variable loss occurred during the subsequent experimental periods. These changes (table 1) were considered to be the result of epinephrine secretion during and following the operative procedures, and this view was substantiated by the finding that such changes never occurred in rats from which the adrenal medullae had been removed prior to the experiments. The average value for initial muscle glycogen was 606 mgm. per cent in a series of adrenal-demedullated eviscerated rats. Changes in blood lactic acid paralleled changes in muscle glycogen. In intact rats blood lactic acid rose from 25 to 50 mgm. per cent in the hour following the evisceration. In the demedullated rats, little or no change occurred if asphyxia or convulsions from hypoglycemia were avoided: in 24 rats of all types (normal or hypophysectomized, with or without glucose) the average increase in blood lactic acid was  $1.4 \pm 1.1^3$  mgm. per cent per hour (table 3).

From these results, it was evident that in order to study muscle glycogen changes resulting from any other causes than glycogenolysis due to epinephrine liberation, adrenal-demedullation previous to other experimental procedures was imperative. Accordingly, all the other animals used in the experiments reported here were so treated, the operation being performed 2 weeks or more

<sup>3</sup> Standard error.



before the experiments or before hypophysectomy, if that was carried out. It is also evident from these data that disappearance of muscle glycogen accompanied by an increase in blood lactic acid, such as is often reported to occur after evisceration or hepatectomy, need have no relation to the normal metab-

TABLE 1  
*Carbohydrate levels in eviscerated rats not given glucose*

	NO. OF OBSERV.	SURVIVAL TIME  minutes	BLOOD SUGAR		MUSCLE GLYCOGEN	
			Initial  mgm. per cent	Decrease  mgm. per cent per hour	Initial  mgm. per cent	Decrease  mgm. per cent per hour
1. Intact rats.....	6	136			440	-147
2. Adrenal-demedullated rats..	12	107 $\pm$ 10.2	82	-37 $\pm$ 3.0	609	+2 $\pm$ 11
3. Hypophysectomized adrenal-demed. rats.....	13	53 $\pm$ 4.5	76	-72 $\pm$ 5.8	529	-108 $\pm$ 16
4. Hypophysectomized adre- nal-demed. rats given A. P. E.*.....	9	89 $\pm$ 5.4	67	-47 $\pm$ 3.6	530	+1 $\pm$ 13

\* Saline anterior pituitary extract, 1 ml. during preceding 24 hours.

TABLE 2  
*Carbohydrate changes in eviscerated rats given glucose*

	CHANGE IN MUSCLE GLYCOGEN DURING GLUCOSE INFUSION (2 HOURS)		CHANGE IN BLOOD SUGAR AFTER CESSATION OF INFUSION	
	No. of observ.	Mgm. per cent	No. of observ.	Mgm. per cent
1. Adrenal-demedullated rats.....	9	+15 $\pm$ 16	12	-33 $\pm$ 4.6
2. Hypophysectomized adrenal-de- medullated rats.....	9	-49 $\pm$ 9	10	-79 $\pm$ 6.1

TABLE 3  
*Blood lactic acid in eviscerated rats*

	NO. OF OBSERV.	INITIAL LEVEL AFTER EVISCERATION	INCREASE DURING EXPERIMENT
		mgm. per cent	mgm. per cent per hour
1. Intact rats.....	6	15.7	+40.
2. Adrenal-demedullated rats.....	16	21.1	+1.3
3. Hypophysectomized adrenal-demedul- lated rats.....	8	16.8	+1.6
Combined data from groups 2 and 3.....	24	19.8	+1.4 $\pm$ 1.1

olism of carbohydrate and should not be confused with the disappearance of carbohydrate due to oxidative processes.

After evisceration, if no glucose was given, the blood sugar fell rapidly in all animals, and hypoglycemic convulsions followed by death occurred when blood

glucose values of 12 to 18 mgm. per cent were approached. The survival time of the animals seemed to be determined primarily by the rate of fall of the blood sugar. As shown in table 1, the survival periods were reduced by nearly one-half by previous hypophysectomy, and the rate of fall of the blood sugar was much faster in this case than in the normal animals. At the same time, also, in the hypophysectomized (adrenal-demedullated) rats, muscle glycogen values fell about 20 per cent, whereas in the control animals no changes occurred. There was no significant increase in the blood lactic acid in either instance. Previous treatment of the hypophysectomized rats with saline anterior pituitary extract lengthened the survival periods to nearly normal duration, and it also prevented the large losses of muscle glycogen. Thus, in the absence of the liver and in the absence of administered glucose, the same kinds of changes in the rate of disappearance of blood and muscle carbohydrate were affected by hypophysectomy and by injection of anterior pituitary extract as are caused by these procedures in intact fasting animals.

The rates at which it was necessary to give glucose to maintain constant blood sugar levels were determined by administering the glucose by constant intravenous infusion over 2-hour periods at various definite rates in different experiments, and determining the blood sugar at half-hourly intervals during this time. The curves obtained in such experiments, plotted as deviations from the initial blood sugar levels, are presented in figures 1 and 2. The average initial blood sugar values, determined immediately before the infusions were begun, were for the normal rats  $84 \pm 1.2$  mgm. per cent (17 expts.) and for the hypophysectomized rats  $79 \pm 2$  mgm. per cent (15 expts.).

From these curves it is apparent that the normal required infusion rates under these conditions lay between 13.5 and 14 mgm. per 100 grams (initial weight) per hour. Rates of from 13.2 to 19.0 mgm. per 100 grams per hour were quite insufficient in the operated rats, the animals going into hypoglycemic shock in 3 of 5 experiments by the end of the infusion periods. The required infusion rates for the hypophysectomized rats were between 25 and 30 mgm. per 100 grams per hour. These rates are comparable to those obtained by Drury and by Greeley in rabbits. They do not represent the maximum utilization rates, but rather those prevailing at blood sugar levels between 75 and 90 mgm. per cent in each case. Since the total oxygen consumption of hypophysectomized rats is about two-thirds that of normal rats, the increased rate of utilization of glucose, if it is oxidized, must represent a very much larger fraction of the total metabolism when the hypophysis has been removed than it does in intact rats.

The rates of fall of the blood sugar after cessation of the glucose infusion were also determined, and were found to be 2 to 3 times as fast in the hypophysectomized rats as in the normal animals (table 2). These figures provide further confirmation of the more rapid rate of removal of the blood sugar observed in the non-infused hypophysectomized rats.

The fate of the glucose disappearing during the infusion cannot be determined directly. In the normal animals, presumably, this glucose, or an equivalent amount of muscle glycogen, is oxidized, for it represents only a fraction of that

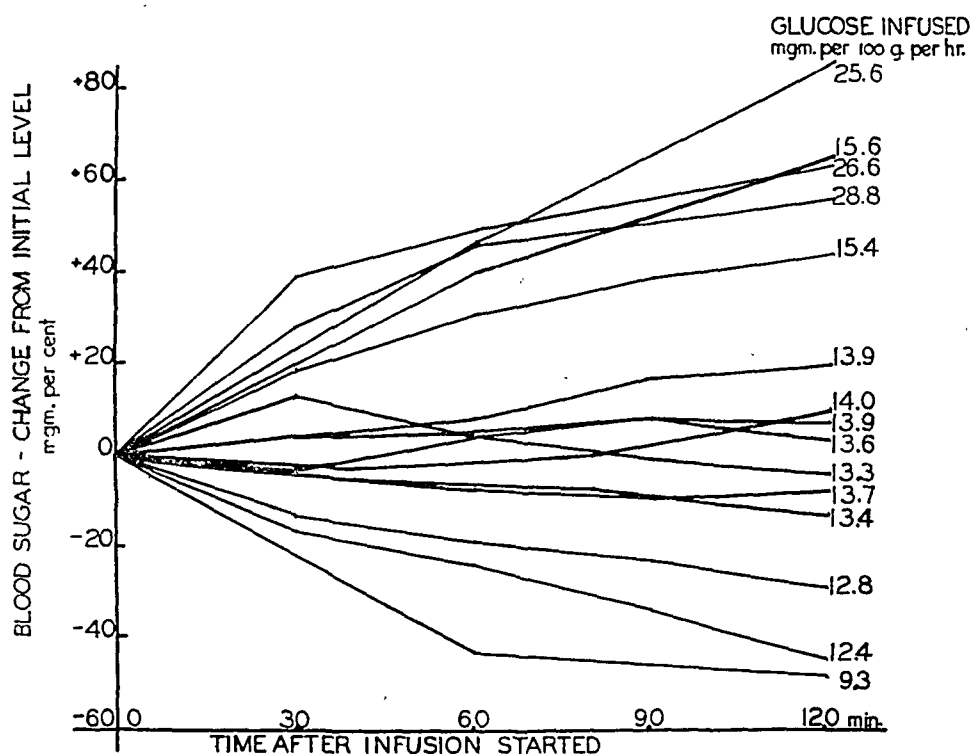


Fig. 1. Maintenance of blood sugar levels in normal eviscerated rats

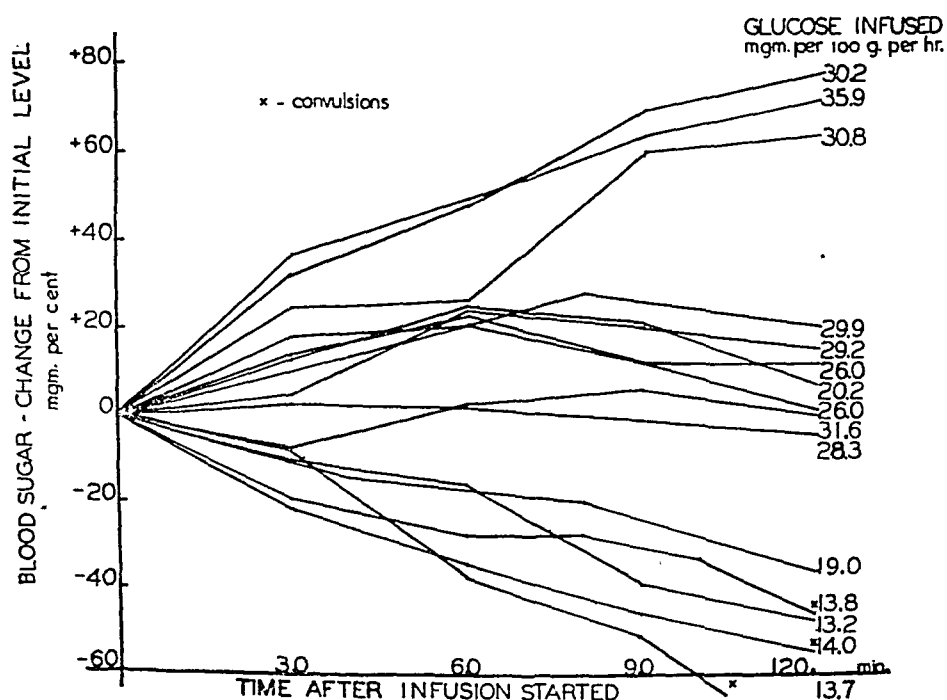


Fig. 2. Maintenance of the blood sugar in hypophysectomized eviscerated rats

required for the total metabolic needs of the animal. The increased rates of disappearance in the hypophysectomized rats were not here due to increased deposition of muscle glycogen, for the gastrocnemius muscle glycogen levels fell slightly during the infusion, while there was no change in the muscle glycogen of the normal rats (table 2). There was no increase in blood lactic acid during the infusion periods, and any large increase in the lactic acid content of the tissues should have been reflected here over the two hours' duration of the experiments. Since these two substances—glycogen and lactic acid—are the only ones in which sufficiently large changes could be expected to occur to account for the disappearance of glucose on any other basis, the carbohydrate, it can be assumed, was oxidized.

One other possible means of carbohydrate disposal remains—that of conversion to fat. However, since the liver is probably necessary in some stages of this

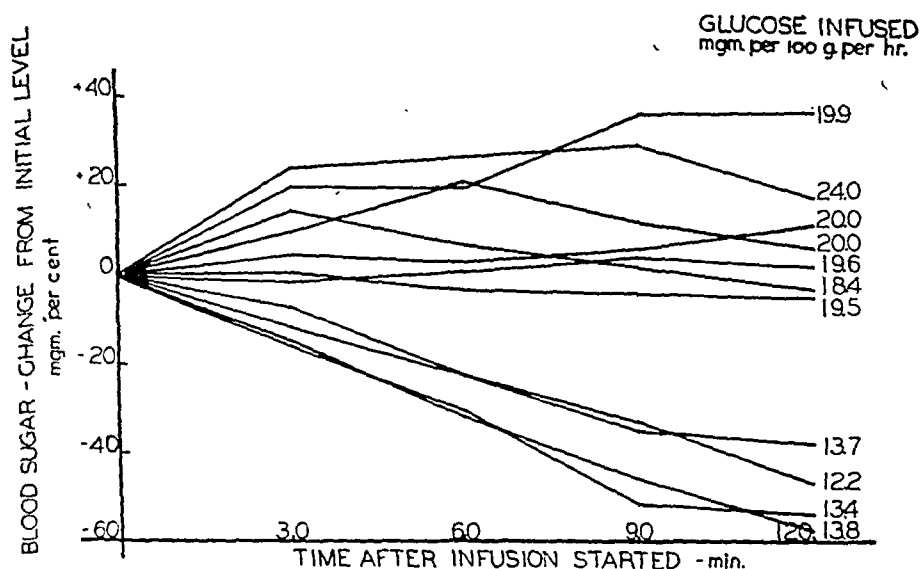


Fig. 3. Maintenance of the blood sugar in nephrectomized eviscerated rats

process, since the blood sugar levels were at no time sufficiently high to make this process likely to be rapid, and since hypophysectomized rats do not show any indications of habitual excessive fat deposition, this conversion remains an improbable explanation of this phenomenon.

The observation of Bergman and Drury (12) that nephrectomy increases the carbohydrate utilization of eviscerated rabbits suggests that defective kidney function in hypophysectomized animals might be operative in producing the results observed. The phenomenon also occurs in rats (fig. 3), but the effects of complete nephrectomy upon the glucose utilization rate were not nearly as great as those of hypophysectomy. The required infusion rate was about 19 mgm. per 100 grams per hour, and the rate of fall of the blood sugar after the cessation of the glucose infusion was  $43 \pm 3.0$  mgm. per cent per hour (9 expts.), a value not significantly greater than the normal rate of fall (table 2).

This effect of nephrectomy on carbohydrate utilization has not been explained. Bergman and Drury suggested that the kidney normally excreted a substance which, if retained, caused increased carbohydrate utilization. However, the removal of the hypophysis, if it affects the secretory function of the kidney at all, can produce only moderate chronic defects. No signs of altered kidney function are obvious in hypophysectomized rats except the diuresis which is large immediately after hypophysectomy and sometimes persists in some degree. Urine excretion often occurred during the course of the infusion experiments and the bladders of the animals were usually full at the end of these periods. There is thus no apparent correspondence between the degree of interference with kidney function and changes in carbohydrate utilization in the two conditions of nephrectomy and hypophysectomy, and it is unlikely that the effects of hypophysectomy can be in any large part due to failure of the excretory function of the kidney.

Another explanation of the effects of nephrectomy on apparent carbohydrate utilization rates is possible: that the kidneys of eviscerated animals may normally be contributing glucose to the blood. It has been observed that kidney slices may synthesize considerable amounts of carbohydrate in the presence of amino acids, and even more from keto acids (13). With 0.025 M 1-(+)-glutamic acid, whole kidney slices from rats formed new carbohydrate at the rate of 4.8 mgm. per gram wet weight per hour. Since there are about 0.85 gram of kidney per 100 grams weight of rat, the kidneys of the intact rat may form glucose from amino acids at the rate of at least 4 mgm. per 100 grams body weight per hour. This is a minimum value because the kidneys *in situ* are very likely more efficient than slices in performing such synthetic processes. It is also of the same order of magnitude as that required to satisfy the difference in the apparent utilization rates of normal and of nephrectomized rats. The blood stream is capable of supplying amino acids in sufficient quantity, since in the eviscerated animal the blood amino nitrogen concentration rises very much above the normal, to levels comparable to those used in the fluid medium of the slice experiments. Thus there is a reasonable possibility that glyconeogenesis in the kidney may play a part in determining the apparent carbohydrate utilization rate in eviscerated animals. No data is available on glyconeogenesis in kidney slices from hypophysectomized rats, but it is probably not less than in tissues from adrenalectomized rats. In the latter case, the total new formation of carbohydrate from amino acids is about 20 per cent below the normal; so that the contribution by the kidneys of the hypophysectomized nephrectomized rats would be but little less—20 per cent of the difference between normal and nephrectomized rats—than that of the kidneys of the normal rats. The large differences in utilization rates of the two series would be accounted for in very small part only by differences in glyconeogenesis by the kidneys.

The present experiments on hypophysectomized rats confirm and extend those of Greeley on rabbits. They are, however, in contradiction to those of Soskin and his colleagues, who worked on dogs (14). The method of measuring carbohydrate utilization used by the latter investigators has been criticized

previously by Chambers and Barker (15) and by Cori and Cori (16). In this method, the decline in body glycogen (calculated from individual muscle glycogen determinations) was added in each case to the amount of glucose infused, and this sum was corrected by the subtraction of increases in the glucose and the lactic acid content of the body (calculated from changes in blood levels). In cases where moderate amounts of glucose were given and blood sugar levels were maintained within normal limits, the major contributions to the final figure obtained for carbohydrate utilization were made by the losses of muscle glycogen. Since these losses were always accompanied by elevation of the blood lactic acid values, they were undoubtedly the result of glycogenolytic processes not necessarily reflecting oxidation of carbohydrate. In the hypophysectomized dogs, Soskin and his co-workers reported that changes in muscle glycogen and blood lactic acid were much smaller than in the normal dog; consequently the utilization figures calculated from these data indicated low or normal utilization rates. Sufficient data are not given to make clear the reason for this difference in glycogenolysis; it may have been due, for instance, to such a non-specific cause as a difference between the two series of animals in their relative ventilation rates, normal dogs requiring more oxygen than the hypophysectomized animals and so exhibiting asphyxial glycogenolysis at ventilation rates which were still sufficient for the operated dogs. In any case, it does not seem profitable to compare utilization rates of carbohydrate calculated upon the basis of changes in muscle glycogen which may not have been related to the oxidation of carbohydrate. It will be necessary to reinvestigate this problem before invoking species differences between rabbits and rats, and dogs, to explain the differences in the apparent effects of hypophysectomy upon the carbohydrate metabolism of these animals.

#### SUMMARY

Normal, adrenal-demedullated and hypophysectomized rats have been compared in their metabolism of carbohydrate in the absence of the abdominal viscera. Removal of the adrenal medullae previous to the evisceration prevented the occurrence of any significant changes in muscle glycogen and in blood lactic acid during or following the evisceration in normal rats, if asphyxia or convulsions were avoided. All animals used in other experiments were then adrenal-demedullated before hypophysectomy or before evisceration was performed, and in these experiments, therefore, glycogenolysis due to epinephrine liberation was avoided.

Hypophysectomized demedullated rats after evisceration survived only half as long as normal demedullated rats and utilized their blood glucose at two to three times the normal rate. Their muscle glycogen also fell moderately during the survival period. This fall was prevented, and the survival time was prolonged, by previous treatment of the rats with saline anterior pituitary extract. There was no increase in blood lactic acid during the survival periods.

Hypophysectomized rats required glucose administration at the rate of 25 to 30 mgm. per 100 grams per hour to maintain normal blood sugar levels, whereas

the control rats required only about 13.5 mgm. per 100 grams per hour. This increased requirement of glucose was not due to deposition of muscle glycogen or to liberation of lactic acid; it was therefore presumed to be due to increased oxidative utilization of carbohydrate in the peripheral tissues, in the absence of the anterior pituitary gland.

Nephrectomy increased moderately the need for glucose in rats, as it does in rabbits. Kidney damage is not indicated to be an important factor in producing the increased utilization of carbohydrate in hypophysectomized rats.

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# HEAT EXCHANGES DURING RECOVERY FROM EXPERIMENTAL DEFICIT OF BODY HEAT

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The adjustments whereby the temperature of the human body remains constant may be considered as consisting in: 1, loss of heat when the temperature is too high, and 2, gain of heat when the temperature is too low. But since heat is continuously both produced and dissipated, net loss may result by slowing the production as well as by accelerating the dissipation. Much evidence indicates that little is done by the overheated man to decrease production, and compensation consists in faster dissipation (Adolph, 1938). What is the situation in the undercooled man? Is body temperature restored by faster heat production (chemical regulation), or by slower heat dissipation (physical regulation), or by both? What part does evaporation of water play in the modification of heat losses?

Quantitative answers to these questions depend on the measurement of changes in heat content of the human body. A reading of temperature in the rectum is sometimes considered to indicate what excess or deficit of body heat exists. It is well known, however, that undercooled parts of the human body are often isolated from interchanging heat with other parts at the usual rates. In the present research the temperatures of many localities are measured, and from them integrated temperatures of the body are estimated. These are converted into equivalent heat contents; thereby the heat content of the body is obtained in units commensurate with those of heat exchange.

PROCEDURE. Several methods of inducing deficits of heat content have been tested. The unanesthetized human body easily defeats most efforts to deplete it of heat. Immersion in cold water was studied calorimetrically by Lefèvre (1911); he showed that the rates of heat loss diminish with each minute of exposure. Exposure to cold air cooled the body somewhat in the tests of Lefèvre, Swift (1932), and others. We found that ice packs, ice-water irrigation of the rectum, and cold shower baths cooled local portions of the body without concurrently diminishing the temperature of the remainder; they did not allow the rapid induction of measurable heat deficits. We therefore drank ice-water.

Body heat production, heat content, heat loss by evaporation of water, and heat loss by radiation and convection were measured during the recovery from

<sup>1</sup> Aided by a grant from the Fluid Research Fund of this School. We are indebted to Dr. S. W. Clausen for facilities and counsel.



each deficit produced. Thus the part played by the several factors in paying off the heat debt could be evaluated at all times during the experiment and could be related to the moving heat debt.

A brick room, insulated on the windowed side by an extra partition of wall-board, was used for the tests. During the experiments the air of this room was kept at  $31^{\circ}\text{C.} \pm 1^{\circ}$  and 20 to 30 per cent relative humidity. The nude subject occupied a reclining seat on a Sauter balance. Rates of non-renal water loss were measured by weighing the man at intervals. Urine collected periodically was kept in a stoppered flask on the balance. Heat production was estimated by means of a Benedict-Roth oxygen consumption apparatus. Rectal temperature was read to  $\pm 0.01^{\circ}\text{C.}$  on a thermometer inserted to a depth of approximately 5 cm., the stem of which projected through a slit in the seat. Readings could thus be taken throughout the test without altering the position of the thermometer. Mouth temperatures were taken by clinical thermometer. Surface temperatures were determined on twelve representative areas with a movable, fine, copper-constantin thermocouple, and then weighted according to the extent of each area represented, to obtain mean surface temperature. The pulse frequency was recorded. Room temperature and relative humidity were ascertained by thermometer and calibrated hair-hygrometer respectively.

The subject entered the room 2 to 5 hours after a light breakfast or no breakfast, undressed and sat on the balance. After 15 to 20 minutes, to allow for partial equilibration of the body with room conditions, a control period of 30 to 50 minutes was inaugurated, during which observations were made at approximately 20-minute intervals. Then the subject drank 1.4 to 1.7 liters of water at  $1^{\circ}$  to  $3^{\circ}\text{C.}$ , taking 10 to 14 minutes for its ingestion, and observations were continued, at as short intervals as possible (15 to 25 min.), for 150 to 210 minutes.

*Heat loss by evaporation.* The weight loss recorded by the Sauter balance represented water evaporated from the body plus carbon lost as carbon dioxide. Since the carbon constituted only 5 to 10 per cent of the total weight loss, no correction for this factor has been made; the total has been treated as loss due to evaporation of water. This water loss has been translated into heat loss by use of the factor 0.58 Cal./gram for latent heat of vaporization of water at  $37^{\circ}\text{C.}$  If a correction for the carbon loss were applied, it would reduce the values for rates of evaporative heat loss by approximately one Calorie per square meter per hour (fig. 1).

The vaporization of water in the control period, while quite variable on different days, accounted on the average for approximately 50 per cent of the heat flowing out of the body. This percentage is probable evidence of slow sweating, at diverse rates. Following ingestion of the cold water, the heat loss by vaporization diminished to only 25 per cent of the total production of heat in the body, a figure which agrees closely with the ratio found by Benedict and Root (1926) and others who studied subjects in cooler environments and in basal states. These minimal rates of vaporization in our experiments prevailed for about 80 minutes after ingestion of the cold water; and the rates on different days agreed closely (fig. 1). By 180 minutes the rates of vaporization had reattained their

pre-ingestion magnitudes. The reduction in rate of loss by vaporization is sufficient to account for 50 to 55 per cent of the heat regained by the body during the 180 minutes of recovery.

Heat production varied as much as 16 per cent from the mean in some subjects, but showed no consistent change after cold water was drunk. It took little, if any, part, therefore, in the recovery from the heat deficit. This conclusion is also suggested by data of Winslow *et al.* (1937) who found no significant alteration in the rate of heat production over a wide range ( $16^{\circ}$  to  $42^{\circ}\text{C}.$ ) of environmental air temperatures, even though large heat deficits (1.0 to 1.5 Cal. per

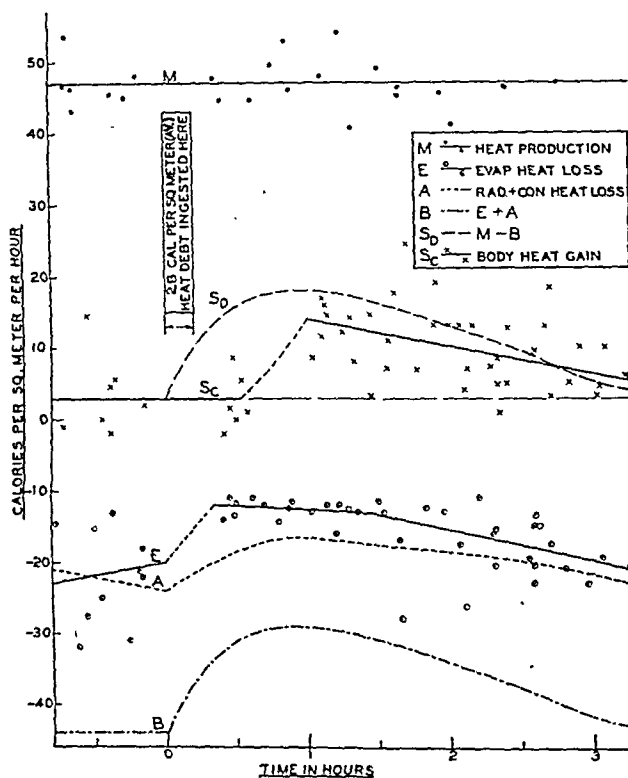


Fig. 1. Body heat exchanges following a heat debt produced by ingestion of 1540 ml. of water at  $1^{\circ}$  to  $3^{\circ}\text{C}.$  in an environmental temperature of  $30.9^{\circ}\text{C}.$  (6 experiments on 4 subjects).

kgm.) resulted when the subjects were exposed to the colder airs. Loewy (1890) and others reported that heat production was not increased by external cooling, except when shivering or other minor muscular activity resulted. Hardy *et al.* (1941) found moderate increases in rate of heat production in women but not in men exposed to cool air.

Cannon *et al.* (1926) in 22 tests on eleven clothed subjects at an environmental temperature of  $20^{\circ}\text{C}.$  found that ingestion of sufficient ice water to produce small heat debts, of approximately 0.45 Calorie per kilogram of body weight, induced an average maximal increase of 10 per cent in heat production in those experiments where shivering was absent. When shivering occurred, brief increases in

heat production up to 90 per cent above basal were recorded. Cannon *et al.* suggested that at higher environmental temperatures such rises in heat production might not be observed.

At the air temperature of 31°C. our rates of heat production varied to the same degree as those of Cannon's subjects who did not shiver. Slight gooseflesh associated with an urge to shiver was felt by two of our subjects. However, actual shivering occurred in only one test; it was slight and of short duration. In the test in which the rate of heat production increased most, the additional heat supplied was sufficient to pay off only 15 per cent of the heat deficit induced.

TABLE 1

Temperature alterations following water ingestion in an environmental temperature of 31°C.

DATE OF EXPT., 1939	SUBJECT	WT.	WATER DRUNK	TEMP. OF INGESTED WA- TER	MAXIMAL TEMPERATURE DEFICIT			CALCULATED DEFICIT IN MEAN BODY TEMP.	RATIO OF DEFICITS OF MEAN SURF. TEMP. : RECTAL TEMP.	TIME AFTER IN- GESTION OF LOWEST RECORDED TEMP.			TEMP. DEFICIT 150 MIN. AFTER INGESTION		
					In rect. temp.	In mean sur- face temp.	In mean body temp.*			In rectal temp.	In mean sur- face temp.	In mean body temp.	In rectal temp.	In mean sur- face temp.	In mean body temp.
Water ingested at 2°C.															
2/1	E. A.	kgm. 76	ml. 1445	3.5	0.90	0.30	0.69	0.72	0.33	68	70	70	0.32	-0.15	0.16
2/6	E. A.	76	1541	2.5	0.73	0.84	0.76	0.80	1.15	56	56	56	0.12	0.57	0.26
1/23	E. P.	65	1710	1.0	1.02	1.49	1.08	1.10	1.44	40	86	48-61	0.25	1.27	0.59
2/13	E. P.	66	1415	1.5	0.72	0.83	0.73	0.87	1.15	42	66	42	0.22	0.07	0.16
2/19	H. K.	79	1649	2.5	0.70	0.41	0.60	0.82	0.59	57	57	57	0.14	-0.15	0.04
2/18	M. P.	72	1482	1.0	0.82	1.10	0.92	0.85	1.34	60	51	51	0.47	0.61	0.54
Average.....			1542		0.81	0.83	0.80	0.86	1.02	54	64	55	0.25	0.37	0.29

Controls—water ingested at 35°C.

2/8	E. A.	76	1527	35.0	0.15	0.16	0.11	0	1.07	67	37	37-67	0	-0.22	-0.08
2/15	E. P.	66	1423	36.5	0.11	0.42	0.20	0	3.85	60	60	60	0.03	0.06	0.04
Average.....			1475		0.13	0.29	0.16	0	2.44	64	48	56	0.01	-0.08	-0.02

\* Calculated from recorded deficits of rectal and mean surface temperatures.

Rectal temperature attained an average maximal decrement of 0.81°C. Fifty-four minutes (average) after cold ingestion were required to reach this maximum, but the time varied as much as 14 minutes from this mean in individual tests. The curves for rectal temperature against time descended smoothly, and subsequently rose gradually, the rate of increase being proportional to the displacement of the rectal temperature below the initial. At the end of 150 minutes after the ingestion, the rectal temperature had recovered two-thirds of this deficit; the average deficit was at this time only 0.25°C. (table 1).

Surface temperature. The mean temperature of the body surface suffered a maximal decrease of 0.83°C. (average), which equals the average maximal dec-

rement of rectal temperature. However, the ratio of maximal decrease of surface temperature to maximal decrement of rectal temperature in individual experiments varied from 0.33 to 1.5 (table 1). The surface temperatures were lowest at 64 minutes (average), or 10 minutes after rectal temperatures were lowest. At 150 minutes the surface temperatures had recovered one half of the deficit and were within  $0.37^{\circ}\text{C}.$  of the initial value. Surface temperatures tended to remain low until the body had recovered some of the heat deficit (table 2). They did not rise at rates strictly proportional to their displacement below the initial, differing from rectal temperature in this respect. Rates of increase of surface temperatures were greatest between 150 and 200 minutes, at a time when rates of increase of rectal temperature had fallen off to relatively small values. By waiting until considerable recovery of rectal temperature had taken place, surface temperatures acted to conserve heat production by reducing heat loss

TABLE 2  
*Rates of temperature change at various times following ice-water ingestion*

MINUTES AFTER INGESTION	TEMPERATURE CHANGE DURING THE INTERVAL, IN $^{\circ}\text{C}.$			RATE OF TEMPERATURE RISE IN $^{\circ}\text{C}.$ PER HR.			PER CENT OF RECOVERY AT END OF EACH INTERVAL		
	Rectal temp.	Mean skin temp.	Mean body temp.	Rectal temp.	Mean skin temp.	Mean body temp.	Rectal temp.	Mean skin temp.	Mean body temp.
0-30	-0.66	-0.59	-0.60						
30-60	-0.15	-0.24	-0.20						
60-90	+0.22	+0.10	+0.15	0.44	0.20	0.30	27	12	19
90-120	+0.20	+0.15	+0.19	0.40	0.30	0.39	52	30	43
120-150	+0.11	+0.17	+0.15	0.22	0.34	0.29	66	51	61
150-180	+0.10	+0.18	+0.12	0.20	0.36	0.24	78	73	76
180-200	+0.04	+0.12	+0.06	0.12	0.36	0.18	83	87	85

Averages of the six experiments at an environmental temperature of  $31^{\circ}\text{C}.$

through radiation and convection. The part played by this lag in the make-up of the temperature deficit will be discussed below.

Oral temperature, which was ordinarily about  $0.5$  to  $0.6^{\circ}\text{C}.$  below rectal temperature, fell sharply when ice-water was taken through the mouth and did not reassume its position relative to rectal temperature until about 60 minutes after ice-water ingestion. Thereafter it rose as the other body temperatures rose.

Decrements of surface temperature tended to be greater on the extremities than on the trunk. Later the surface temperature of the extremities increased more slowly than that of the trunk region; thus the surface temperatures of arms and legs were the last to recover.

*Mean body temperature and heat content.* The mean body temperature was derived from measurements of the rectal temperature and mean surface temperature with the use of the following formula (Burton, 1935):  $\frac{2}{3}$  rectal temp. +  $\frac{1}{3}$  mean surface temp. = mean body temp. Thus computed, the average maximal fall of the body temperature was  $0.80^{\circ}\text{C}.$ , while the average time that it took this fall to occur was 55 minutes. This represents the time for the heat

deficit, contained in the water ingested, to become evenly distributed through the body. Sixty per cent of this deficit had been recovered at 150 minutes (table 2), and 85 per cent at 200 minutes.

The amount by which the mean body temperature would have decreased if the distribution of the heat debt through the whole body had taken place immediately and equitably, is calculated in the following manner:  $\Delta T = W(T_b - T_w) / (W + 0.83 B)$ , where  $T_b$  is the initial mean body temperature,  $T_w$  is the temperature of the water on its ingestion,  $W$  is the amount of water ingested in kilograms,  $B$  is the body weight in kilograms before ingestion, and 0.83 is the specific heat of the body.  $\Delta T$  thus calculated for individual experiments is recorded in column 9 of table 1.

Interestingly enough, the calculated temperature fall exceeds but little the actual fall found 55 minutes after the beginning of ingestion (column 8). Only 7 per cent of the heat debt seems to have been paid off within this period of time.

*Heat loss by radiation and convection* was computed as follows. *a.* Total heat loss equalled heat production minus heat retained or stored. Total heat loss minus evaporative heat loss was then heat loss by other means (radiation, convection, conduction). Alternatively, *b.* the heat loss by these other means during the pre-ingestion period having been ascertained, this fraction of heat loss in the subsequent periods was assumed to be directly proportional (Gagge, 1936; Winslow *et al.*, 1937) to the difference between mean surface temperature and air (and wall) temperature. Such calculations show that 9.0 Calories per square meter per hour flowed from the body for each 1°C. difference between skin and environment, during the pre-ingestion period of these experiments. (This figure is 9.4 if we correct the evaporative heat loss for the carbon exhaled as previously discussed.) Winslow, Herrington and Gagge's (1937) data show a radiation and convection loss rate of only 7.0 Calories per square meter per hour per degree C. difference between skin and environment. This discrepancy of 2.0 or 2.4 Cal. is most likely due to a difference in convection in the two experimental conditions. Turbulent air movement in their experiments was 5 to 6 meters per minute; air movement of 14 meters per minute in our experiments would account for the discrepancy noted. While we did not measure air movement, the velocity mentioned is considered likely.

*Total heat exchanges.* In figure 1 the sum of heat loss by vaporization and of heat loss by radiation and convection (*b*) is plotted as total heat loss. This total heat loss when subtracted from heat production gives the curve for heat storage in the body, designated by  $S_D$ . Another curve for heat storage, designated  $S_C$ , is a mean of the rates of heat gain by the body as indicated by the rise in mean body temperature during intervals in each test. The points plotted between 0 time and 60 minutes, a period when the mean body temperature was actually falling, however, were determined by subtracting the recorded fall in body temperature from the fall in body temperature ( $\Delta T$ ) which the water as ingested was calculated to produce (table 1).

The average heat debt induced in the body computed from  $\Delta T$  was approximately 53 Calories. The debt paid off under curve  $S_D$  represented approxi-

TABLE 3

*Recovery from a deficit of body heat in an environmental temperature of 28°C.*

TIME	RECTAL TEMP.	MEAN SKIN TEMP.	MEAN BODY TEMP.	MEAN BODY TEMP. DEFICIT	HEAT PRODUCTION	NON-RENAL WEIGHT LOSS	URINARY EXCRETION RATE
	°C.	°C.	°C.	°C.	Cal./sq. m./hr.	gm./hr.	ml./hr.
7:50- 9:38							28
9:16- 9:43						56.6	
9:23					40.0		
9:30	37.48	33.50	36.15				
9:43-10:04						56.6	
9:48					43.7		
9:52	37.50	33.36	36.12				
10:04-10:54						36.1	
10:10					40.0		
10:15-10:41	Ingested 1468 ml. of water at 1.0°C.						
10:20	37.42			(0.08)			
10:40	37.22			(0.28)			
10:43	37.16	32.59	35.64	0.48			
10:54-11:12						35.1	
10:57	36.83			(0.67)			
10:58					38.1		
9:38-11:14							82
11:04	36.75			(0.75)			
11:12-11:31					38.9		
11:14	36.68			(0.82)			
11:14-11:36							493
11:18					39.4		
11:31-12:01						30.3	
11:36	36.61			(0.89)			
11:36-12:08							510
11:49	36.62	32.42	35.22	0.90	41.8		
12:01-12:26						37.6	
12:03	36.71	32.45	35.29	0.83			
12:14					40.0		
12:08-12:29							565
12:18	36.80	32.55	35.38	0.74			
12:26-12:56						33.7	
12:29-12:48							538
12:33	36.90	32.46	35.42	0.70			
12:48- 1:02							407
12:48	36.90	32.38	35.39	0.73			
12:56- 1:14						31.4	
1:03	36.91	32.57	35.46	0.66			
1:02- 1:23							300
1:16	36.90	32.70	35.50	0.62			

Subj., A. W. Wt., 69 kgm. S. A., 1.85 sq. meters. Aug. 9, 1939. Expected deficit of mean body temperature ( $\Delta T$ ), 0.88°C.

mately 73 Calories. These computations suggest that the decrease in total heat loss after ingestion is somewhat exaggerated by curves  $S_D$  and B, and that the heat exchange represented by curve  $S_C$  is the more accurate one.

In general, the deficit of heat was replaced in the body not by augmented production of heat through oxidative metabolism, but through diminished loss of heat. Half the diminution was in evaporative loss and half was in radiative loss. Recovery was half complete in 120 minutes and wholly complete in approximately 220 minutes after the deficit arose.

In control experiments, in which water at average body temperature was ingested, slight decreases in mean body temperature ( $0.11$  to  $0.20^{\circ}\text{C}.$ ) resulted. The heat deficit produced in this manner was completely paid off in 150 minutes after drinking (table 1).

*Pulse frequency.* Following ingestion of cold water the pulse frequency decreased sharply, reaching its minimum between 20 and 30 minutes after drinking began. From about 9 beats per minute below its initial frequency, the rate gradually increased until at 180 minutes it was only 4 beats per minute below the initial (fig. 2). In the control experiments the pulse frequency also diminished, but more gradually and less.

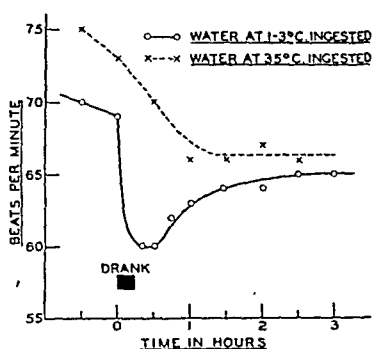


Fig. 2. Change in pulse rate after ingestion of 1540 ml. of water. (Averages of 5 + 2 experiments on 3 subjects.)

The sharp decline in pulse frequency following ice-water ingestion may be due to conduction between the large mass of cold water in the stomach and the heart. The position of the stomach when filled with cold water, could be felt as a cold area on the external abdominal wall.

No correlation between pulse frequency and oxidative metabolic rate could be noted.

*Rate of urine excretion.* The diuresis which resulted from the ingestion of 1540 ml. of water was sometimes delayed when the water was taken cold (fig. 3). In one subject the diuresis appeared 65 and 90 minutes later than it did after a control ingestion of the same amount of water at  $35^{\circ}\text{C}.$  (E.F.A., fig. 3). The maximal rates of urine excretion after cold water ingestion were often less than after an equal amount of water at  $35^{\circ}\text{C}.$  was drunk. Thus the ingestion of cold water diminished the maximal rate of excretion as well as delayed the diuresis; both are phenomena which might represent deferred absorption of the cold water from the alimentary tract.

*Effect of air temperature on recovery from body heat deficit.* One experiment was

conducted in air at 28°C., in the same manner as those at 31°C. previously described. A condensed protocol of the results (table 3) shows that recovery from the heat deficit at 28° is much slower and less complete than in the warmer air. In 180 minutes after ingestion of the water only 31 per cent of the maximal deficit had been paid off, and recovery as indicated by rectal temperature was at a standstill. In an equivalent length of time in 31°C., 76 per cent of the deficit was recovered and all temperatures were still rising (table 2).

At environmental temperatures higher than 31°C. an induced heat deficit might be discharged more rapidly than at 31°C., since current loss of heat could be still further diminished.

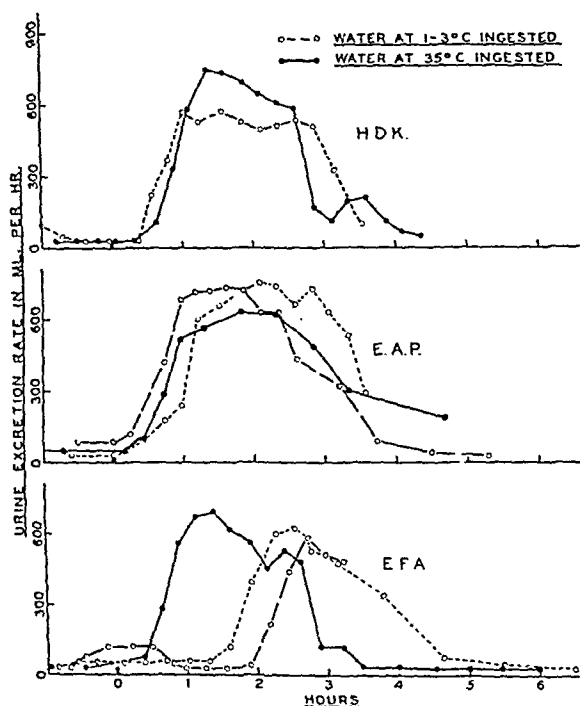


Fig. 3. Diuresis produced by ingestion of 1540 ml. of water, begun at zero time and completed within 10 to 14 minutes. Note delay in excretion in last subject occasioned by ingestion of cold water (broken-line curves).

Since even in air at 28°C. no significant alteration in heat production was found after ingestion of the cold water, all the recoveries here recorded are to be attributed to decrease in the rate of heat loss. In other subjects, differently acclimatized, an increase of heat production might be found.

*Comment.* Having been taught that extra heat production (chemical regulation) occurred when the human body was too cool, we were interested to find that in the conditions of our experiment such was not the case. Instead, heat loss was reduced so that no extra energy was expended in making up the heat deficit.

The occurrence of "chemical" regulation in man has been demonstrated by several early workers (Yagloglou, 1924). Hardy *et al.* (1941) showed that women exposed to cold air produced heat appreciably faster; men did not. But re-



covery from cooling has been studied only in the discontinuous measurements of Cannon *et al.* (1926), who found extra heat production in several subjects; the significant increments seem to have accompanied shivering.

Possibly shivering and augmented heat production occur more often with cool skin. Our adult subjects did not compensate for heat deficit by augmenting heat production; the same individuals may have had the ability to do so in infancy, and may be able to acquire it again after acclimatizing exposures. If chemical regulation acts in more extreme deficits of heat, or in deficits of particular kinds, or in certain states of acclimatization, these contingencies need to be demonstrated for man.

#### SUMMARY

Heat exchanges were measured in four men who sat unclothed in air at 31°C. and 25 per cent relative humidity. In each test the body became cooler by 0.72 Calories per kilogram when 1.5 liters of ice-water were drunk. The mean heat content was estimated from the temperatures of many regions of the body at various times thereafter.

In 200 minutes, 85 per cent of the initial heat debt was paid off. Half of the heat regained came from the reduction in loss by vaporization of water. The other half came from the reduction in loss by radiation and convection, concomitant with the diminution of surface temperatures.

Heat produced by oxidations was not augmented. No shivering occurred. At a lower environmental temperature (28°C.) the recovery was slower.

It was noted that the heart frequency was diminished after ice water was drunk, and that the ensuing water diuresis was sometimes delayed.

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# PRESSURE OF BLOOD IN THE RIGHT AURICLE, IN ANIMALS AND IN MAN: UNDER NORMAL CONDITIONS AND IN RIGHT HEART FAILURE<sup>1</sup>

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It has long been known that the pressure of blood in the right auricle of a normal anesthetized mammal, lying supine, varies around a mean value slightly less than zero or atmospheric (1, 2). The pressure in the great veins, as would be expected, shows a gradual increase as one passes peripherally.

There has been, however, little study either of the right auricular or of peripheral venous pressures in animals in conditions of right heart failure.

No measurements of right auricular pressures have previously been recorded in man, either under normal conditions or in heart failure, so far as we are aware.

I. *Venous and auricular pressures during right heart failure in animals.* Right heart failure in an animal was first encountered accidentally. Pressures were being recorded by means of saline manometers in arm, leg, and right auricle of a large chimpanzee. These results are shown in figure 1. After the experiment had proceeded for about an hour and a half, a needle was inserted into the pleural space to obtain a record of pleural pressure. The animal rapidly developed pulmonary edema. At the same time both auricular and peripheral venous pressures rose and both became nearly equal. These phenomena clearly indicated an acute heart failure, the rise of venous pressure indicating the degree of "backward failure."

Autopsy of the animal showed moderate pulmonary edema. There was no evidence of injury to the lung from the intrapleural needle.

A study of the same phenomena was subsequently undertaken, using dogs. The technique was similar except that right auricular pressure was recorded by long rubber catheters passed into the right auricle either from below via femoral vein or from above via the jugular.

A preliminary question concerned the reliability of the technique of using long catheters of narrow caliber (no. 6 to no. 10) passed along the great veins, for recording of central venous pressure.

a. It was easily shown that a catheter could be passed into the femoral vein up

<sup>1</sup> A preliminary report of a part of the material was presented at the Association of American Physicians, May, 1941.

<sup>2</sup> Working under a grant from the Commonwealth Fund.

the vena cava to the auricle, and withdrawn again, with no measurable effect upon the pressure in jugular vein, in right auricle (as measured by another catheter extending down from the jugular vein), or in the opposite femoral vein. There appeared, therefore, to be no effects due to obstruction or spasm of vessel walls.

b. There was no significant error due to capillary effects within the catheter. The levels of both saline manometers were too high by 4 mm., by reason of the capillary effect at the meniscus. Correction was made for this error.

c. The changes in pressure with the animal's respiration, however, were not recorded accurately by the long intra-auricular catheters. This was to be expected, since to record such changes involved fairly rapid flow of fluid through the catheter, with each inspiration and expiration.

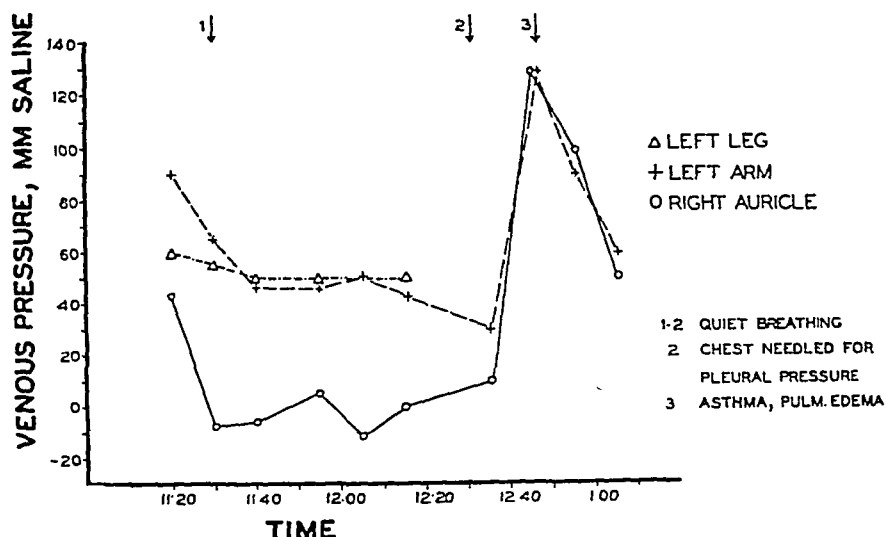


Fig. 1. Venous pressures, in millimeters of water, in the arm, leg and right auricle of an anesthetized (nembital) chimpanzee.

The actual expiratory levels of venous pressure could be obtained separately, simply by shutting off the connection between catheter and manometer during inspiration, opening it only during the phase of expiration. Equilibrium was established slowly in this way, after perhaps a dozen respirations. Shutting off the connection during expiration similarly gave the inspiratory venous pressure level. It was found that the pressure recorded when the manometer was left connected continuously with the right auricle was always the lowest or inspiratory pressure, saline being more readily drawn down the catheter during inspiration, than blood returned into it during expiration.

Figure 2, describing one of several similar experiments on dogs, gives the essential results. It will be seen that the deep labored respiration of tracheo-bronchial obstruction increased greatly the pressure differences between inspiratory and expiratory states, in the right auricle.

For practical purposes it was found that with dogs anesthetized by nembital,

the use of an intratracheal cannula prevented obstructive respiration and maintained inspiratory-expiratory pressure differences near minimum values.

For the study of right heart failure (associated with acute pulmonary edema), we used the technique of Miller and Matthews (3), producing pulmonary edema

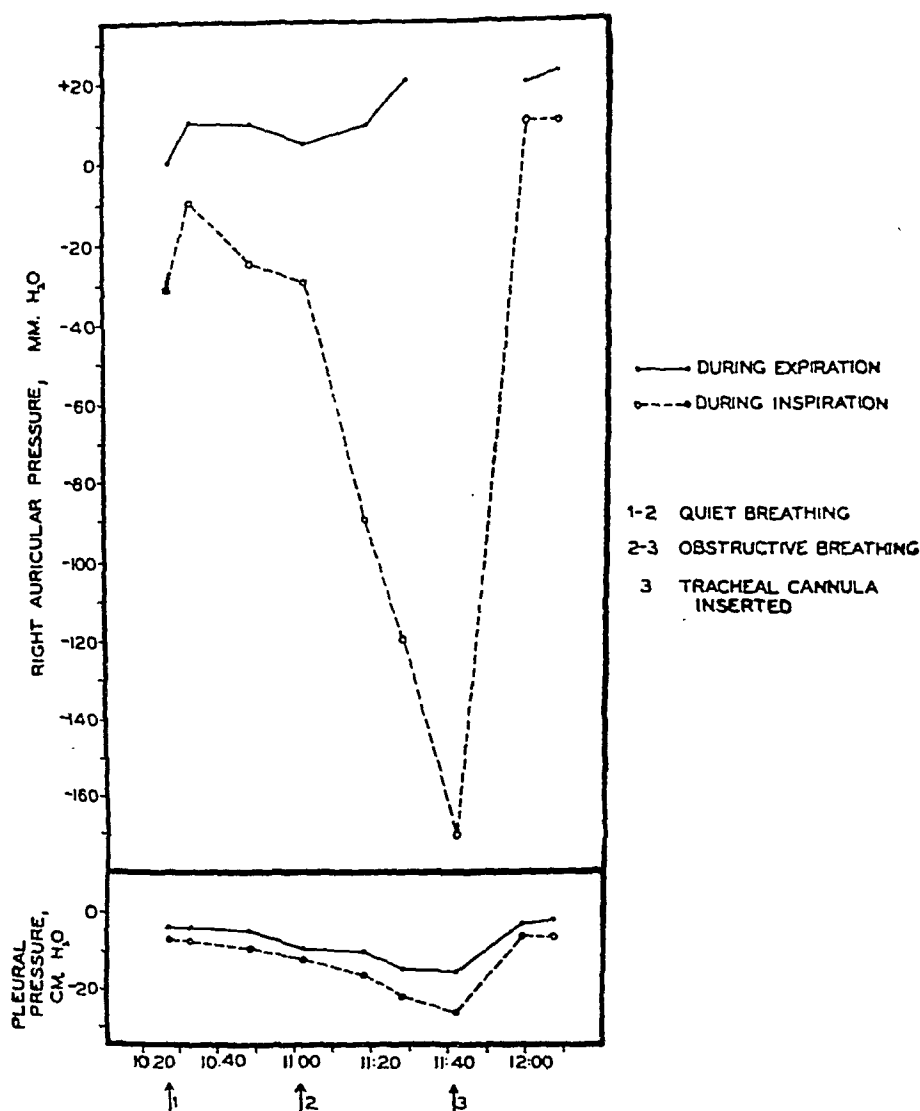


Fig. 2. Anesthetized dog (nembutal) showing the effects of spontaneously developing respiratory obstruction, upon the inspiratory and expiratory phases of right auricular and pleural pressures.

by inhalation of ethyl acetate vapor. Figure 3 gives the results of four such experiments.

It was found that by adjusting the concentration of vapor inhaled, pulmonary edema could be brought on relatively rapidly—in 15 to 30 minutes—or very gradually. In the third experiment shown in figure 3, a low concentration of ethyl acetate vapor was inhaled continuously for 83 minutes.

All animals at autopsy showed dilated right auricles and pulmonary edema, the latter demonstrated grossly and microscopically. Dogs 4, 5 and 6 died as a direct result of the ethyl acetate administration; dog 7, given the lower concentration of the gas, while still in a relatively early stage of right heart failure, and with arterial pressure maintained, was killed by intracardiac chloroform.

The chief finding in these experiments confirms that of the chimpanzee experiment; namely, that with development of right heart failure both peripheral

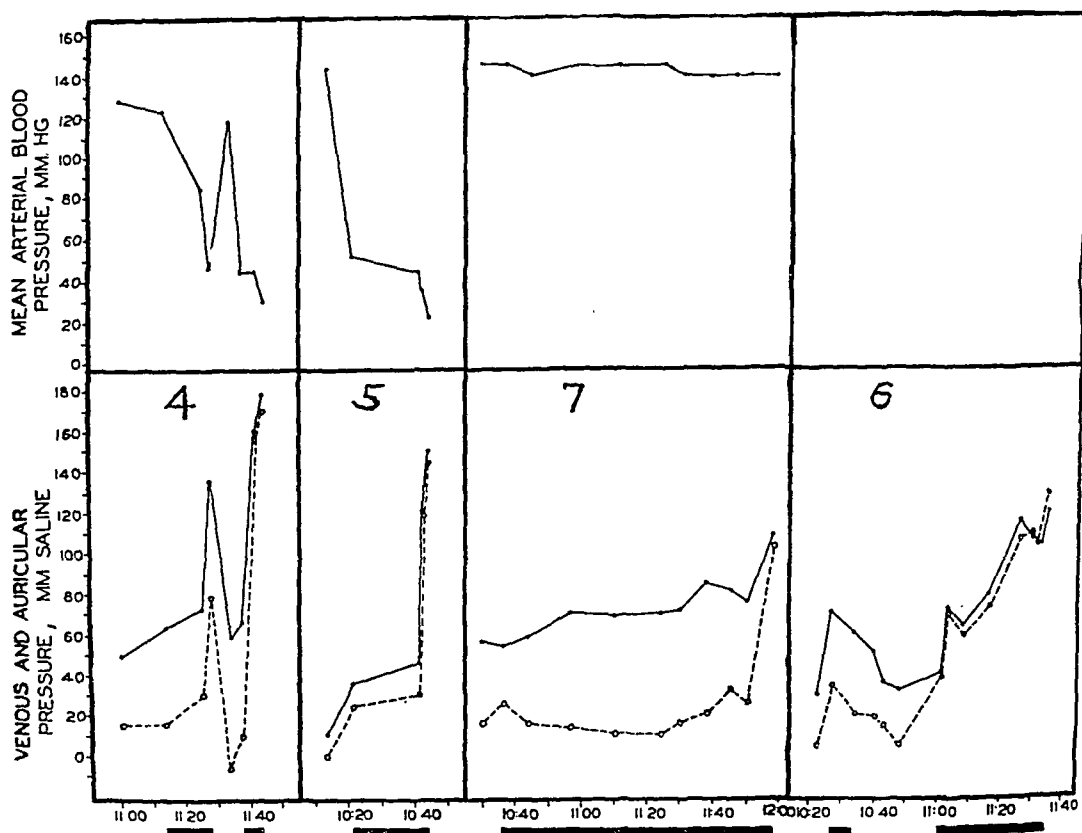


Fig. 3. Effects of inhalation of ethyl acetate vapor, upon arterial, venous, and right auricular pressures, in a series of 4 dogs. Upper curves: mean arterial pressures. Lower curves: pressures in femoral vein (solid lines), and right auricle (broken lines). Solid blocks below indicate periods during which ethyl acetate was inhaled.

and central venous pressures rise and the gradient between peripheral and central pressures decreases almost to the vanishing point.

II. *Pressure in the right auricle of man, under normal conditions and in right heart failure.* Catheterization of the right heart in man was first essayed by Forssmann (4), and has subsequently been used for various studies (5, 6, 7, 8, 9, 10). The usual technique is to insert a long (70 cm.) ureteral catheter (no. 7 or no. 8 French) into a median basilic vein, then pass it along brachial, axillary, and subclavian veins until (under fluoroscopic observation) the tip is seen to lie in the right auricle. Cournand and Ranges (11) have recently developed improve-

ments in certain details of this technique, and have used right heart catheterization in man for determination of cardiac output by the direct Fick principle. These investigators showed that right heart catheterization caused but slight changes in circulation, respiration, and respiratory exchange; notably, a slight increase in minute ventilation and a slight slowing of the pulse rate.

In the present study, we have used the technique of right heart catheterization for recording venous pressure, in nine normal subjects and in six patients with heart disease, three of whom were in congestive failure, the other three recovering from this condition. The general procedure was as follows:

The preparation of the arm and the insertion of the catheter were carried out with the subject lying in his bed. When the catheter had been passed successfully into the axillary vein, and a slow flow of saline (15 drops per min.) from a reservoir was established through the catheter to maintain its lumen and prevent clot formation, the subject was transferred to the fluoroscopy table, and the catheter then pushed on into the right auricle under fluoroscopic observation. Anteroposterior and lateral x-rays were usually taken. By these x-rays, a record of the catheter's position was made, and the absolute level of the catheter in reference to the outer chest wall could be determined. The subject then was transferred back to his bed, supine with head very slightly elevated, and the catheter was connected with a saline manometer. Peripheral venous pressure was recorded in the usual manner by the direct method, with a needle inserted in a vein of the other arm, and connected with a second manometer. The zero of both manometers was taken arbitrarily as a point 5 cm. below the angle of Louis on the external anterior chest wall. In the cases where x-rays of the catheter were made, the manometer figures were subsequently corrected according to the actual position of the catheter in the chest, as will be discussed later.

A series of observations of peripheral venous and of right auricular blood pressures was then carried out. On several occasions, the catheter was withdrawn by stages, thus affording opportunity to measure pressures in superior vena cava, subclavian and axillary veins. The gradient of pressure appeared to be nearly linear, as might be expected. It should be noted that during such withdrawal of the catheter, there was no change in the pressure recorded in the vein of the opposite arm, thus indicating that the procedure itself probably does not disturb venous pressure levels.

The respirations of the subjects, both normal and cardiac, were quiet and easy during the procedure. It did not seem likely that there would be large inspiratory-expiratory differences in auricular pressure. In two subjects, expiratory pressures were recorded separately, by shutting off the manometer during each inspiratory phase. It was found that there was less than 10 mm. difference between inspiratory and expiratory pressures, and this factor was therefore disregarded, in this series.

The results of all observations of peripheral and central pressures are shown in table 1.

It will be seen that the peripheral pressures of normal subjects varied from 102 mm. to 56 mm.; auricular pressures from 61 mm. to 8 mm. The gradients varied

from 76 mm. to 12 mm., with a mean of  $+41$  mm. As a matter of fact, in this group of subjects with apparently normal circulations, all arm-to-heart pressure gradients were 25 mm. or over, except for one case. This was a man of 56, recovering from pneumonia. He was being allowed up in a chair, still had a mild tachycardia at the time of the examination. Whether this case should be considered as having a normal circulation, or whether he should be placed in the group of "subnormal circulation" as found by Starr (12) in post-infectious conditions, is perhaps debatable.

The absolute value of right auricular pressure, in the six cases where the position of the tip of the catheter was determined by lateral x-ray, varied from 61

TABLE 1

*Pressures in right auricle and in arm vein, in subjects with normal circulation, and with congestive heart failure*

SUBJECT	AGE	DIAGNOSIS	VENOUS PRESSURE, MM. H <sub>2</sub> O	
			Arm	Right auricle
C. N.....	51	Ca. of sigmoid	75	45
J. Ri.....	38	Ca. of bladder	70	8
C. C.....	60	Renal tuberculosis	63	38
J. Re.....	73	Arteriosclerosis	59	23
F. K.....	41	Normal	56	21
P. T.....	47	Normal	76	26
G. T.....	62	Ca. of liver	102	26
D. P.....	56	Pneumonia	73	61
G. P.....	43	Lead poisoning	81	49
Average.....			74	33
L. L.....	59	Arteriosclerotic heart	214	216
G. B.....	47	Chronic nephritis	128	128
		(later, failure more advanced)	182	184
M. H.....	32	Constrictive pericarditis	291	285
A. R.....	56	Coronary thrombosis	78	71
W. D.....	67	Coronary thrombosis	48	31
J. W.....	42	Rheumatic heart	61	40

mm. to 8 mm., with a mean of  $+37$  mm. This is of interest, since most textbooks assume the blood pressure in the right auricle of man to be approximately zero. This value has evidently been taken over from animal data. In the supine position in man, the heart tilts sharply upward,—the more so the deeper the chest,—so that the tip of the right ventricle is well above the right auricle. A zero or negative auricular pressure would be unfavorable for adequate ventricular filling, though of course such filling would be aided in so far as the negative intrathoracic pressure is effective within the heart chambers.

Another point to be mentioned is what *part* of the relatively roomy right auricle the tip of the catheter actually rested in. As measured in our lateral x-rays, in most cases the catheter tip lay about 2.5 cm. from the posterior margin of the

heart shadow, which would be perhaps slightly posterior to the exact middle of the right auricle.

Turning now to the measurements of peripheral venous and auricular pressures in cases of heart disease, we find a striking contrast to the values in normal subjects (table 1). In all four observations on patients in congestive failure, the elevated venous pressures were associated with a sharp decrease or practical disappearance of peripheral-central pressure gradients, auricular and arm pressures being nearly identical. The phenomenon is apparently the same as that described above in animals with right heart failure associated with acute pulmonary edema.

In the three measurements on cardiac patients recovering compensation (table 1), peripheral-central pressure gradients appeared in two to be within limits of normal; in the third, the gradient of only 7 mm. was less than normal.

DISCUSSION. In normal subjects, though the gradients of pressure from arm to heart are somewhat variable, the mean value of +41 mm. of water is similar to that observed between extremity and right auricle in animals.

This gradient must depend largely upon two factors, the size of the venous channels from arm to heart, and the amount of blood flow through them; the arm-to-heart pressure difference being greater, the larger the blood flow and the narrower the venous channels.

It would be expected that under the conditions of heart failure, with dilated peripheral veins and decreased total blood flow, the arm-to-heart pressure differences would decrease; and this is strikingly shown in the actual measurements in congestive failure. There must have been in all cases some excess of peripheral over central pressure, in order for any blood flow to have continued; but in three of the observations this gradient was so small that it was not detectable in the measurements obtained.

Finally, a point of some interest, on which these data offer a very limited amount of information, is the adequacy or inadequacy of various venous pressure reference points that have been suggested as the "zero," or position of the right auricle within the chest. This subject has recently been well presented by Lyons, Kennedy and Burwell (13), and need not be reviewed here.

Figure 4 gives the difference between the actual position of the catheter tip in the right auricle (represented by the horizontal zero line), and the position of the right auricle as it would be estimated, in each of six cases, by the use of various reference points. It will be seen that for these cases, the reference point of Eyster and that of Lyons, Kennedy and Burwell, are closest to the actual position of the right auricle. Other reference points, however, are grouped well together. If, for example, an arbitrary zero level were taken 7 cm. below the angle of Louis, there would be, for these six cases, a good approximation of the actual position of the catheter in the right auricle.

While varying gradients of venous pressure between arm and heart make any peripheral pressure measurement inaccurate as a measure of right auricular pressure, even if the position of the latter is accurately known, it is nonetheless important from the practical standpoint to have some approximately valid



anatomical reference point for venous pressure measurement. With the accumulation of sufficient data, of the type given in figure 4, this could readily be determined.

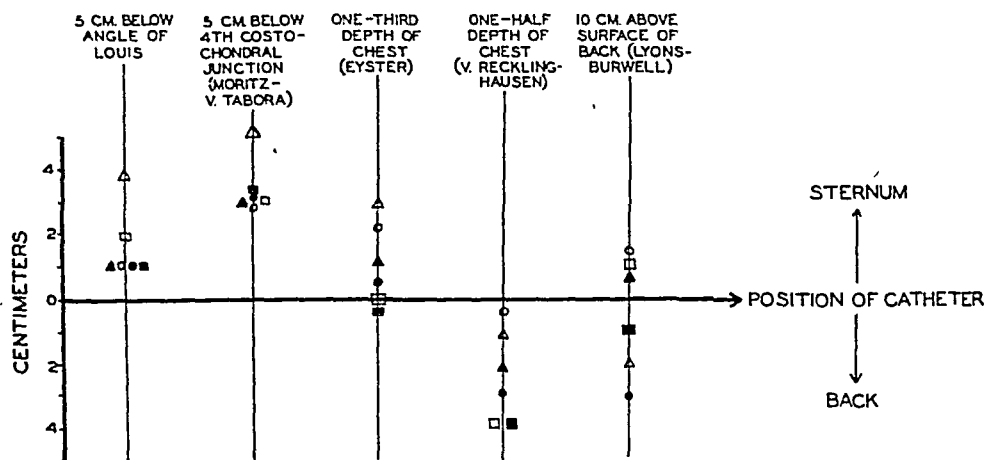


Fig. 4. Relation of actual position of catheter in the right auricle, to the supposed position of the right auricle as estimated by various investigators, in 6 normal human subjects.

#### SUMMARY

1. In a study of venous pressures in one chimpanzee and a series of dogs, the development of right heart failure associated with acute pulmonary edema was accompanied not only by rise of peripheral and right auricular pressures, but also by a disappearance of the normal pressure gradient between peripheral veins and right heart, the right auricular and peripheral venous pressure levels becoming nearly equal.

2. In nine human subjects with apparently normal circulations, right auricular pressure was recorded directly by means of right heart catheterization. The average gradient from arm to heart was +41 mm. of water. In six subjects absolute pressure levels at the right auricle were determined by locating the position of the catheter by lateral x-ray; the average right auricular pressure (subjects supine) was +37 mm.

3. Three patients in congestive heart failure, with high peripheral venous pressures, showed decrease in peripheral-central pressure gradients, the pressures in arm vein and in right auricle being almost identical.

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# THE INTERRELATIONSHIP OF p-AMINOBENZOIC ACID AND INOSITOL

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The discovery of the chromotrichial action of p-aminobenzoic acid by Ansbacher (1) precipitated a controversy as to the true nature of the grey hair factor or factors. Unna et al. (2) and Emerson (3) failed to find any chromotrichial action attributable to p-aminobenzoic acid and believe that pantothenic acid is the only grey hair factor. Martin and Ansbacher (4) noted a chromotrichial action of p-aminobenzoic acid in mice. It seemed obvious that some factor must be at play not recognized by either group of investigators. To bring these various observations into harmony has been the objective of this work.

**PROCEDURE AND RESULTS.** The basic diet used throughout these experiments consisted of:

	per cent
Vitamin free casein.....	18.0
Sucrose.....	67.0
Salts.....	4.0
Butter fat.....	9.0
Cod liver oil.....	2.0

The diet was made up each week, and the vitamin supplements were added as follows:

	mgm. per kilo of diet	daily dose per rat
Thiamine hydrochloride.....	5.0	50 µg.
Riboflavin.....	10.0	100 µg.
Pyridoxine.....	5.0	50 µg.
Nicotinic acid.....	100.0	1 mgm.
Calcium pantothenate.....	100.0	1 mgm.
Choline chloride.....	200.0	2 mgm.
Inositol.....	200.0	2 mgm.
p-Aminobenzoic acid.....	100.0	1 mgm.

Each experimental group consisted of 30 Rockland strain black rats.

**Diet 1. Inositol and pantothenic acid deficiency:** This diet contained six factors but was deficient in pantothenic acid and in inositol. On this combined deficiency, the Rockland strain black rat attains a weight of approximately 55 to 65 grams and death occurs within five or six weeks. Spectacled eyes are seen within two weeks. The involvement of the eyes develops to a pan-ophthalmitis. This is bilateral and severe. Purulent exudate, bloody exudate, rupture of

eyeballs and complete blindness characterize the syndrome. It is far more marked than we have noted in a straight pantothenic acid deficiency or in a straight inositol deficiency. There is some alopecia of a very mild variety. Greying or achromotrichia is not a pronounced manifestation. The animals are a brownish grey. The fur has a wet, oily appearance. These animals before death invariably show a peculiar gait; the hind legs are handled awkwardly and do not seem to respond in coördination with the forelegs. Complete paralysis was not seen in any of the thirty animals in this group but fully 90 per cent showed this spasticity.

*Diet 2. Inositol deficiency:* This diet contained seven of the factors listed and was deficient in inositol. The growth rate of this set is such that after four or five weeks the average weight is 70 to 90 grams. At five weeks the animals show a pattern greying which is markedly similar to that of a pantothenic acid deficiency. Spectacled eyes are common, with pan-ophthalmitis occurring infrequently and not to the marked degree seen in a combined inositol and pantothenic acid deficiency. Again, in this group the locomotor incoördination occurs, but to a lesser degree; and the mild alopecia observed might be called rather a thinning of the hair than real alopecia. The syndrome associated with inositol deficiency is difficult to distinguish from that of a pantothenic acid deficiency.

*Diet 3. p-Aminobenzoic acid deficiency:* Eighty per cent of the animals were dead within six weeks. The weights of this set reached 50 to 60 grams. The appearance is most unusual: the hair over the body is sleek and black and appears darker than its original color; the hair over the head is sparse, with a mild greying. Crusted, bloody, scaly feet were frequently seen. These animals seem to have no energy; they move sluggishly with hunched backs.

*Diet 4. Pantothenic acid deficiency:* No deaths occurred in this group during the first eight weeks. The weights attained averaged 70 to 100 grams. A typical pattern greying was seen, accompanied by the usual spectacled eyes. A mild form of alopecia was noted. Pan-ophthalmitis was seen in but two of the thirty rats on this pantothenic acid deficient diet.

*Diet 5.* This diet contains the eight members of the B complex listed. At six weeks, the weight attained averaged 110 to 180 grams. The animals seemed normal in every respect except that the coat was rather brownish. This diet was supplemented with wheat germ oil, ethyl linoleate and biotin, and found to be complete as evidenced by a failure of biotin or the essential unsaturated fatty acids to increase the growth rate.

*Diet 6.* This diet contained no inositol and no p-aminobenzoic acid, but did contain the other six factors as listed. These rats do not differ in any way from those on diet 5. The weight, the appearance, in every respect, they are the same. Thus, in the absence of both inositol and p-aminobenzoic acid, the six factors, namely, thiamine, riboflavin, pyridoxine, choline, nicotinic acid and calcium pantothenate are adequate.

**DISCUSSION.** The results of Unna et al. (2) and Emerson (3) are confirmed in that the six basic factors (thiamine, riboflavin, nicotinic acid, pyridoxine, choline and calcium pantothenate) are adequate for seemingly normal nutrition.

They are not adequate if either p-aminobenzoic acid or inositol is added to the diet. Ansbacher (1) and Martin and Ansbacher (4) had inositol in their basic supplements and thus p-aminobenzoic acid deficiency is noted. Neither Unna et al. (2) nor Emerson (3) had inositol in the basic diet used. It is possible that the explanation lies in a stimulation and/or inhibition of bacterial growth in the intestinal tract and hence the bacterial synthesis of vitamin factors, known or unknown in nature. Both factors, namely, inositol (5) and p-aminobenzoic acid (6, 7), have been demonstrated as growth factors for yeast or bacteria, and p-aminobenzoic acid has been demonstrated to inhibit bacterial growth (8) at certain concentrations. The utilization and consequent destruction of various vitamins by microorganisms has been demonstrated (9, 10). Further, the synthesis of certain factors, particularly biotin (11) and inositol (12) by organisms present in the gastro-intestinal tract has been reported. The stimulation of the growth of microorganisms by one member of the B complex causing an increased synthesis of another member of the B complex by that same organism is not only probable but certain. The analysis we place on the above reported results is that inositol stimulates the growth of organisms which utilize and destroy some member of the B complex, known or unknown, thus precipitating a deficiency of that factor. p-Aminobenzoic acid either through stimulation or inhibition of bacterial growth precipitates an inositol deficiency (4). This seems the logical explanation, in view of the fact that the elimination of both inositol and p-aminobenzoic acid from the diet, feeding only six of the B complex factors results in a state of nutrition which is the equal of that noted if the eight factors are included.

The addition of inositol to a diet may stimulate the growth of organisms which destroy pantothenic acid, whereas the addition of p-aminobenzoic acid may inhibit the growth of these organisms. This explanation can be advanced even if inhibition is not considered, as specific stimulation may cause an overgrowth of one type of organism and the consequent elimination from the gastro-intestinal tract of another. In the preliminary experiments, it is our opinion that proteus vulgaris is absent from the gastro-intestinal tracts of rats fed excessive amounts of p-aminobenzoic acid (5 mgm. daily). Further, we have noted a tendency on the part of the acid forming organisms to overgrow. Thus, we see both an apparent inhibition of proteus and a stimulation of the lactic acid forming organisms. The seeming inhibition of proteus may be actually due to overgrowth on the part of the acid forming organisms.

It is not desired to suggest an alteration of the status of either inositol or p-aminobenzoic acid as a member of the B complex, but to point out that one precipitates a deficiency of the other under our experimental conditions.

The addition of p-aminobenzoic acid to the basic diet made it possible for Martin and Ansbacher (4) to produce an inositol deficiency, where in the absence of p-aminobenzoic acid no inositol deficiency developed (13). Woolley, who originally described this syndrome (14), stated subsequently that in many instances spontaneous cures occurred (15). It is these spontaneous cures which are prevented by the p-aminobenzoic acid. The cures were due to synthesis of

inositol by organisms present in the gastro-intestinal tract (12), and it seems probable that it is this synthesis which is prevented by p-aminobenzoic acid.

#### CONCLUSIONS

Six B complex factors (thiamine, riboflavin, pyridoxine, choline, nicotinic acid and calcium pantothenate) afford seemingly normal nutrition to the Rockland strain black rat. Addition of inositol precipitates a syndrome prevented by p-aminobenzoic acid. Addition of p-aminobenzoic acid precipitates a syndrome prevented by inositol. Eight B complex factors (the six listed above plus inositol and p-aminobenzoic acid) afford seemingly normal nutrition to these rats.

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# INFLUENCE OF ANTERIOR PITUITARY EXTRACT ON PROTEIN AND CARBOHYDRATE METABOLISM

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The anterior pituitary gland is known to act as an anabolizer of protein.

Treatment of rats (7) and dogs (1, 2, 18) with anterior pituitary extracts containing growth hormone results in decrease of N excretion. The N.P.N., Urea N and Amino N of the blood is decreased in dogs, rats and guinea pigs (19, 1, 14). The N.P.N. and its constituents in the tissues are decreased. At the same time the total N content of the tissues increases, thus indicating an increase in protein N (8). Mirsky (13), on the basis of his experiments on depancreatized and on eviscerated dogs, came to the conclusion that the anabolism of protein following injection of anterior pituitary extracts is due to increased insulin output. Anterior pituitary extract increases protein breakdown. But in presence of the pancreas the latter is stimulated by a concomitant pancreatotrophic action of the anterior pituitary extract to increased insulin output. The latter in turn anabolizes protein, thereby not only compensating, but overcompensating the direct protein-catabolizing action of the pituitary extract. The net result in the intact animal, therefore, is protein anabolism or protein "sparing."

The following experiments were performed in order to compare the action of anterior pituitary extract on protein and carbohydrate metabolism. Some parallelism should be expected if the anabolism of protein were an indirect action due to increased insulin output. It was, furthermore, to be examined whether either of the actions on protein and carbohydrate metabolism is dependent on the adrenals or the pancreas. A brief preliminary report on these studies has appeared (15).

**METHODS.** Adult male white rats were used. Animals were fed Purina dog chow, with lettuce given twice weekly. They had free access to tap water. Adrenalectomized animals received 1 per cent sodium chloride solution instead of tap water. Pancreatectomized animals were fed a liquid diet<sup>2</sup> twice daily by stomach tube. Since other studies, to be reported elsewhere, were performed on them, they were kept in individual metabolism cages and their urines were collected in 24 hour periods.

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<sup>2</sup> *Diet.* The diet consisted of: Starch, 540 grams; Dextrin, 260 grams; Sucrose, 265 grams; Egg albumin, 200 grams; Salt mixture, 40 grams (McCollum-Davis N-185) (5); Cellu flour, 120 grams; Vi-Penta, 10 cc.; Wheat germ oil, 10 cc.; *Mazola* oil, 10 cc.; made up with water to 2000 cc.

The anterior pituitary extract used was Antuitrin G (Parke, Davis & Co.).<sup>3</sup> The extract was injected intraperitoneally.

Blood samples were drawn by heart puncture. No anesthetic was used. Blood was immediately pipetted into test tubes containing the appropriate protein precipitants without adding any anticoagulant.

Urea N was determined by the method of Rappaport and Glaser (17) with one minor modification—N/100  $\text{H}_2\text{SO}_4$  was used as a recipient instead of hypobromite and N/100 NaOH was consequently used for titration, neutral red serving as indicator (16). Two-tenths cubic centimeter of blood was used for every determination.

Blood sugar was determined by Benedict's micromethod, using 0.1 cc. of blood for every determination (5).

Adrenalectomy was performed under ether anesthesia through the usual dorsal incision. Animals were used for experiments 5 to 6 days after operation. During this period they received the laboratory stock diet, but with 1 per cent sodium chloride solution instead of tap water.

Partial pancreatectomy was performed under ether anesthesia in young rats weighing about 80 grams.<sup>4</sup> Animals were used for experiments only several months later after they had reached a weight from 150 gams to 250 grams. During this time they received the laboratory stock diet. After they had reached full weight, they were put in individual metabolism cages and received forced feeding as mentioned above.

All animals were fasted for about 16 hours prior to the experiments.

**RESULTS.** *Group 1—normal rats.* Twenty adult male rats fasted for about 16 hours received 1 cc. of Antuitrin G intraperitoneally. Blood was drawn before, and 3 and 6 hours after injection. The urea N content of the blood decreased in all experiments, the decrease varying from 10.5 mgm. per cent to 3.5 mgm. per cent (average 7.4 mgm. per cent). The blood sugar decreased in 13 of the 20 animals (65 per cent), in 6 the blood sugar remained constant and in 1 there was a marked rise. Arbitrarily in these and the following experiments changes of blood sugar of less than 10 mgm. per cent were considered as within limits of possible normal fluctuation. Only changes of 10 mgm. per cent or more were considered significant.

There is no parallelism of the degree of decrease of Urea N and of decrease of blood sugar. Thus in rat 67 the Urea N decreased 10.5 mgm. per cent (from 13.6 to 3.1 mgm. per cent). There was no decrease of blood sugar. In rat 68 the Urea N decrease was 10.1 mgm. per cent (from 13.6 mgm. per cent to 3.5 mgm. per cent) and the blood sugar decreased 49.5 mgm. per cent (from 84.5 to 35.0 mgm. per cent).

*Group 2—adrenalectomized rats.* Twenty-four adrenalectomized male rats

<sup>3</sup> Antuitrin G used in these experiments was generously supplied by Parke, Davis & Co. through the courtesy of Dr. E. A. Sharp.

<sup>4</sup> The author is indebted to Dr. D. J. Ingle for kindly demonstrating the method of partial pancreatectomy. The method employed is in all important points identical with that published since by Harrison and Long (4).



fasted for about 16 hours were studied after intraperitoneal injection of 1 cc. of Antuitrin G in the same way as were the normal animals. Decrease of Urea N varied from 14.7 mgm. per cent to 5.3 mgm. per cent (average 8.2 mgm. per cent). Decrease of blood sugar was found in 13 of 24 animals (54 per cent). Again there appeared to be no correlation of drop of blood sugar to that of Urea N.

As is indicated by the normal range of fasting blood sugars and Urea N values, none of the animals was in a condition of severe adrenal insufficiency. They were adequately maintained through sodium chloride, although some of them lost weight between operation and experiment. Most of the animals died in the days following the experiment, after withdrawal of sodium chloride. Completeness of adrenalectomy was checked by autopsy. However, no search was made for extra adrenal cortical tissue.

*Group 3—partially depancreatized rats.* Thirteen experiments were performed on 9 rats. Urea N content of the blood decreased in all experiments, varying from 11.9 mgm. per cent to 3.8 mgm. per cent (average decrease 8.4 mgm. per cent). Blood sugar decreased in 6 of 13 experiments (46 per cent). This group is much less uniform than are the two former, inasmuch as the degree of pancreatic insufficiency and consequently the severity of the diabetic condition varied to some extent. Five animals had only occasional mild glycosuria and no glycosuria on the day preceding the experiment. Two of these 5, however, had a moderate fasting hyperglycemia. Three of the 9 rats later received stilbestrol as diabetogenic agent (6). In all 3 stilbestrol aggravated the diabetes considerably. These 3 animals were tested for their reaction to the pituitary extract before and after administration of stilbestrol.

*Rat P-1* received a total of 1.9 mgm. Stilbestrol subcutaneously in oily solution over a period of 4 days. No glycosuria was observed before stilbestrol treatment and a glycosuria up to 830 mgm./24 hours in the period after treatment. A single injection of 1 cc. Antuitrin G in the period before treatment resulted in decrease of Urea N of 5.6 mgm. per cent. The blood sugar did not change. The same experiment performed 4 days after stilbestrol treatment (glycosuria on the day preceding the experiment 780 mgm. per cent, on the day of experiment 396 mgm. per cent) produced an increase of blood sugar of 72.9 mgm. per cent. The Urea N decreased 12.9 mgm. per cent.

*Rat P-2* received a total of 1.9 mgm. Stilbestrol in oily solution subcutaneously over a period of 4 days. Glycosuria before stilbestrol treatment varied from 20 to 100 mgm. per 24 hours. After treatment glycosuria reached a maximum of 630 mgm./24 hours. A single injection of Antuitrin G in the period before treatment resulted in decrease of Urea N of 4.9 mgm. per cent. The blood sugar increased 12.4 mgm. per cent within 6 hours. Unfortunately no 3 hour sample was taken.

The same experiment 4 days after Stilbestrol treatment produced a decrease of Urea N of 7 mgm. per cent. Blood sugar did not change during the first 3 hours and rose 40 mgm. per cent after 6 hours. Glycosuria on the day preceding the experiment was 630 mgm./24 hours.

Rat P-3 received a total of 7.8 mgm. Stilbestrol in oily solution subcutaneously over a period of 11 days. No glycosuria was observed before stilbestrol treatment. After treatment there was glycosuria up to 470 mgm./24 hours. Injection of 1 cc. of Antuitrin G during the period prior to stilbestrol treatment was followed by a decrease of Urea N of 3.8 mgm. per cent and a decrease of blood sugar of 19 mgm. per cent during the first 3 hours and of 29.6 mgm. per cent during the 6 hour period.

One day after Stilbestrol treatment the experiment was repeated. Urea N now decreased 10.9 mgm. per cent, blood sugar decreased 13 mgm. per cent during the first 3 hours and returned to the fasting level of 92 mgm. per cent after 6 hours. On the day preceding the experiment there was glycosuria of 340 mgm.

TABLE 1

TYPE OF EXPERIMENT	BLOOD SUGAR						UREAN					
	Fasting		3 hours after inj.		Difference		Fasting		6 hours after inj.		Difference	
	Mean	$\sigma^*$	Mean	$\sigma^*$		$\sigma_{\text{Diff.}}^\dagger$	Mean	$\sigma^*$	Mean	$\sigma^*$		$\sigma_{\text{Diff.}}^\dagger$
Normal.....	88.5 (13)†	12.32	60.0	15.78	28.5	5.19	12.4 (11)	2.48	5.1	2.4	7.3	1.04
Adrenalectomized.....	86.8 (13)	10.53	67.0	12.06	19.8	4.44	13.3 (15)	2.99	5.1	2.05	8.2	0.94
Pancreatectomized .....	118.4 (6)	20.14	93.6	11.52	24.8	9.48	13.0 (13)	2.48	4.6	3.58	8.4	1.20

$$*\sigma = \sqrt{\frac{\sum(\bar{x})^2}{n-1}}$$

$$\dagger\sigma_{\text{Diff.}} = \sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}} \quad (\text{A. B. Dill: Principles of medical statistics, 1937.})$$

‡ Figures in brackets indicate the number of experiments, from which means have been calculated.

It is apparent that neither the magnitude of the blood Urea N decrease nor the blood sugar response following injection of Antuitrin G is correlated to the severity of the diabetes, the severity being judged by glycosuria or fasting hyperglycemia.

*Statistical evaluation.* In table 1 statistical evaluation of the foregoing data is given. The means and standard deviations for the blood sugar values have, of course, been computed from the experiments only which showed hypoglycemia following injection of the pituitary extract. It can be readily seen that the drop of blood sugar is significant, since the difference (fasting blood sugar — 3 hour value) is more than 5 times  $\sigma_{\text{Diff.}}$  in the normal rats and more than 4 times  $\sigma_{\text{Diff.}}$  in the adrenalectomized animals. The pancreatectomized rats show a much greater variation since, as mentioned above, the group included diabetes of

varying severity. Consequently, the difference (fasting blood sugar -3 hr. value) is only 2.3 times  $\sigma_{\text{Diff}}$ .

The decrease of Urea N is uniform in all three groups, the difference (fasting Urea N -6 hour value) is significant, its value being 7 to 8 times  $\sigma_{\text{Diff}}$ .

In table 2 the correlation of changes of blood sugar and of changes of Urea N has been computed. All experiments are included in this table. Since the blood sugar in some experiments did not change, and in some showed a rise, the mean blood sugar decrease of all experiments is considerably less than the mean of the hypoglycemic values as computed in table 1. The correlation coefficient is low. The independence of the blood sugar changes and of the Urea N changes is evident.

DISCUSSION. Anterior pituitary extracts are known to induce nitrogen retention as indicated by increase of total nitrogen in the tissues (8), decrease of N excretion in the urine (1, 2, 7) and decrease of N.P.N., Urea N and Amino N in the blood (1, 14, 15, 19). Decrease of Urea N was used as indicator of the action of the pituitary extract on protein metabolism in the present experiments. Using a micromethod, 0.4 cc. of blood only was needed for duplicate determina-

TABLE 2

	BLOOD SUGAR DECREASE $\bar{M}$	BLOOD UREA N DECREASE $\bar{M}$	$r^*$
	<i>mgm. per cent</i>	<i>mgm. per cent</i>	
Normal. ....	-21.4	-7.3	0.46
Adrenalectomized. ....	-12.4	-8.2	-0.13
Pancreatectomized. ....	-7.1	-8.4	-0.25

$$* r = \frac{M(x \cdot y) - (Mx) \cdot (My)}{\sigma_x \cdot \sigma_y}$$

tions of Urea N and additional 0.2 cc. for duplicate blood sugar determinations. It was thus possible to study the blood chemistry in short term experiments of 6 hours.

Mirsky (13) has concluded from his experiments both on depancreatized and on eviscerated dogs that the anterior pituitary gland enhances protein breakdown. This action, however, is masked in the intact animal by a concomitant pancreatotrophic action of anterior pituitary extract. This leads to increased insulin output and the insulin in turn acts as anabolizer of protein.

Anterior pituitary extracts have been found to decrease the blood sugar levels of dogs (20) and of rats (4). Weinstein working on dogs in short term experiments used the same extract, Antuitrin G, as did the present author. Harrison and Long treated their rats for two and three days with two types of extracts, one a saline extract, the other an alkaline extract prepared after Burns and Ling.

These findings of a decrease of blood sugar following treatment with anterior pituitary extracts would seem to support Mirsky's views. However, in the experiments reported in this paper a decrease of blood sugar which might be considered indicative of increased insulin output was found in only 65 per cent

of the normal animals treated with anterior pituitary extract whereas the Urea N values were decreased in all animals irrespective of the presence or absence of a drop of blood sugar.

If the protein anabolism were due to an increased insulin output stimulated through a pancreatotrophic action of the anterior pituitary extract, blood sugar changes should be roughly parallel to the changes in nitrogen metabolism. The lack of such parallelism makes the above mentioned theory unlikely.

In the experiments of Harrison and Long (4) both alkaline and saline pituitary extracts lowered the blood sugar of fasting rats. The saline extracts also lowered the N.P.N. and reduced the urinary nitrogen excretion whereas the alkaline extracts failed to exert any detectable effect on protein metabolism. This observation would seem to indicate as do our experiments that the actions of anterior pituitary extracts on protein metabolism and on carbohydrate metabolism are not necessarily linked.

Moreover, adrenalectomized animals are known to be more sensitive to insulin. Consequently, if the pituitary extract acted by stimulating insulin output, the effect of the induced hyperinsulinemia should be more marked in adrenalectomized animals. However, the blood sugar drop occurred in about the same percentage of adrenalectomized rats (54 per cent) and normal rats (65 per cent). The difference of 65 per cent normal and 54 per cent adrenalectomized rats showing a drop of blood sugar can hardly be considered significant considering the number of experiments performed (20 normal, 24 adrenalectomized rats). But if anything it would rather tend to show a greater number of normal rats developing hypoglycemia. Also the actual drop of blood sugar was not more marked in adrenalectomized than in normal rats. On the contrary the average blood sugar decrease of the 13 normal rats was 29.2 mgm. per cent as against an average fall of blood sugar of 19.7 mgm. per cent in 13 adrenalectomized rats. The difference of these two values is  $9.5 \pm 4.3$ . The significance is doubtful since the value of the difference is only slightly more than  $2\sigma$  but again, if anything, it would tend to show a lesser degree of hypoglycemia in the adrenalectomized rather than in the normal rats.

In all animals, both normal and adrenalectomized, there was a decrease of Urea N, indicating the action of the anterior pituitary extract on protein metabolism. The decrease of Urea N was present whether or not the blood sugar was lowered. And in those experiments in which a decrease of blood sugar did occur the magnitude of this decrease did not parallel the magnitude of decrease of Urea N. The same holds true also for the third group of experiments, those on partially depancreatized rats. The decrease of Urea N was present whether or not the blood sugar decreased. Moreover, both the changes of blood sugar and those of Urea N seemed to be independent of the severity of the diabetes. It should, however, be noted that none of the rats was very severely diabetic. In 2 of the 13 experiments on pancreatectomized rats the blood sugar increased following the injection of the extract, in one as much as 72.9 mgm. per cent, in the other 9.1 mgm. per cent. However, a rise of blood sugar was also observed in one experiment on normal rats (no. 74) and in one adrenalectomized animal

(no. 58). Gaebler and Galbraith (3) found the same extract, Antuitrin G, severely diabetogenic in completely depancreatized dogs. Of course, we do not wish to imply that an occasional rise of blood sugar in the acute experiments is to be considered as a diabetogenic effect. In three partially depancreatized rats Stilbestrol was given as a diabetogenic agent. Confirming the results of Ingle (6), glycosuria increased considerably. These three rats were tested before and after Stilbestrol administration; in other words, in a stage of mild diabetes and again in a stage of more severe diabetes. These experiments also failed to give any indication that either the decrease of Urea N or the hypoglycemia following injection of Antuitrin G is correlated to the severity of the diabetes.

It is known that the diabetogenic action of certain anterior pituitary extracts is mediated in part at least through the adrenal cortex and that pituitary extracts are not diabetogenous in the absence of the adrenal cortex (11, 12). Young (21) has suggested the hypothesis that the growth hormone acts as such including its protein anabolizing action as long as the pancreas is intact. When the islet response is impaired the same hormone is diabetogenic. It may be of importance in this respect to note that in contradistinction to the diabetogenic effect the protein anabolizing effect is not mediated through the adrenals. As has been shown in this paper, this effect is equally present in intact and in adrenalectomized rats. This result is in accord with that of Harrison and Long (4).

The decrease of blood sugar induced by anterior pituitary extract also is independent of the adrenals. This fact not only raises doubt as to the pancreatic origin of this hypoglycemia, as has been mentioned above; it also makes it difficult to interpret the blood sugar lowering effect and the diabetogenic effect as being two phases of one and the same factor or process.

The fact that both hypoglycemia and decrease of blood Urea N following anterior pituitary extract injection in partially depancreatized rats is independent of the severity of the diabetes also militates against the assumption of a mediation of the anterior pituitary effects through the pancreas. However, since we know nothing of the reactivity of the pancreatic remnants, the experiments on partially depancreatized rats are not conclusive.

#### SUMMARY AND CONCLUSIONS

1. An anterior pituitary extract containing growth hormone (Antuitrin G) regularly produced a decrease of blood Urea N, indicative of protein anabolism, in the normal fasted rat. In 67 per cent (13 of 20 expts.) there was a concomitant decrease of blood sugar. However, the drop of Urea N was present whether or not the extract produced hypoglycemia. The magnitude of the decrease of blood sugar and blood Urea N were not correlated.

2. In the adrenalectomized fasting rat maintained with sodium chloride, the pituitary extract produced the same decrease of Urea N. The hypoglycemia following injection of Antuitrin G was neither more frequent nor more marked

in the adrenalectomized rat than in the normal. The hypoglycemia was perhaps less marked, but the significance of the difference is doubtful.

3. In the partially pancreatectomized, diabetic rat the decrease of Urea N and the hypoglycemia induced by Antuitrin G did not differ from those in the normal animal. The changes in these blood constituents did not seem to be correlated with the severity of the diabetes in the individual animal.

4. These results would indicate that the enhancing effect of anterior pituitary extracts on protein anabolism is not mediated either through the adrenals or through the pancreas. It also is improbable that the hypoglycemia induced in a number of animals by administration of anterior pituitary extract is due to the increased output of insulin, since adrenalectomized rats would be expected to be more sensitive to an excess of insulin than normal animals.

5. The growth hormone is generally supposed to be the factor responsible for the changes in protein metabolism. The possible bearings of the results enumerated above on the relationship of the growth hormone to the diabetogenic factor of the anterior pituitary gland are briefly discussed.

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# THE INFLUENCE OF DIETHYL-STILBESTROL ON CARBOHYDRATE METABOLISM IN NORMAL AND CASTRATED RATS<sup>1</sup>

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In earlier experiments it was shown that the administration of stilbestrol caused an elevation of carbohydrate levels in the intact rat (1, 2). The naturally occurring estrogens have also been shown to raise carbohydrate levels (2, 3, 4). Further evidence of a relation between sex hormones and carbohydrate metabolism is found in the observations that variations in carbohydrate stores are present in animals of different sexes (5, 6) and after castration (7).

In the experiments to be reported at this time a study has been made of the effect of stilbestrol on castrated rats. In addition, further studies have been made on normal animals.

Thirty-one castrated and 26 normal rats received subcutaneous injection of 100 gamma of diethyl-stilbestrol<sup>2</sup> daily for periods of 5, 10 or 20 days. Castrations were performed at least three weeks before treatment was begun. Twelve castrate and 14 normal uninjected animals were used as controls. All animals were fasted for 36 hours before killing and urine was collected in sulphuric acid during the last 24 hours of the fast. As in our earlier studies (1) determinations were made for blood sugar, liver- and muscle-glycogen, and urinary nitrogen. The gonads, adrenals and pituitaries were weighed and together with the mammary glands were prepared for microscopic examination.

The blood sugar levels were variable as is indicated by their standard deviation (table 1). However, the figures for these probably are within the normal range for rats fasted 36 hours, except in the case of the animals treated for 20 days. In the latter instances the blood sugar levels of the females (table 2) showed an appreciable elevation. It is our experience that the blood sugar level will rise for 5 or 6 hours after an injection of stilbestrol. The same phenomenon has been noted by Zunz and Labarre (8) after the administration of estradiol benzoate. However, the acute effect was eliminated in the present experiments since the rats received their last injection on the day before they were sacrificed.

Deuel, Gulick, Gruenwald and Cutler (6) found that intact female rats which had been given specific quantities of glucose showed higher blood sugar levels

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<sup>2</sup> Diethyl-stilbestrol was supplied to us through the kindness of Dr. Richard Johnson of the Frederick Stearns Company and Dr. J. A. Morrell of E. R. Squibb & Sons.

than males treated in a similar fashion. In the present experiments only the females which had been treated for 20 days showed an appreciable difference in blood sugar values. This rise was less striking in the male animals which had

TABLE 1

*Averages of normal and castrated rats treated with 100 gamma stilbestrol daily*

NUMBER OF ANIMALS	TREATMENT	WEIGHT AT END OF 36-HOUR FAST	BLOOD SUGAR	GLYCOGEN		URINARY N LAST 24 HOURS	WEIGHT OF PITUITARY	WEIGHT OF ADRENALS (2)	WEIGHT OF OVARIES (2)	WEIGHT OF TESTES (2)
				Liver	Muscle					
		gm.	mgm. per cent	mgm. per cent	mgm. per cent	mgm./gm. body wt.	gm./100 gm. body wt.	gm./100 gm. body wt.	gm./100 gm. body wt.	gm./100 gm. body wt.
14	Normal	167	69 $\pm$ 4*	83	323	0.826	0.0047	0.0249	0.0400 (8)†	1.359 (6)†
12	Normal castrated	217	71 $\pm$ 8*	115	342	0.868	0.0052	0.0189		
8	Normal 5 day stilbestrol	163	72 $\pm$ 8*	239	293	0.748	0.0077	0.0357	0.0503 (3)†	1.270 (5)†
9	Castrated 5 day stilbestrol	206	75 $\pm$ 8*	285	331	0.694	0.0068	0.0304		
10	Normal 10 day stilbestrol	149	74 $\pm$ 8*	331	311	0.774	0.0083	0.0346	0.0537 (6)†	1.122 (4)†
9	Castrated 10 day stilbestrol	192	73 $\pm$ 7*	323	355	0.812	0.0084	0.0410		
8	Normal 20 day stilbestrol	131	84 $\pm$ 6*	409	260	1.042	0.0110	0.0550	0.0595 (8)†	
13	Castrated 20 day stilbestrol	188	83 $\pm$ 11*	594	354	0.960	0.0109	0.0407		

\*Standard deviation.

†Numbers in parentheses are number of animals.

TABLE 2

*Averages of normal and castrated male and female rats treated with 100 gamma stilbestrol daily*

NUMBER OF ANIMALS	TREATMENT	SEX	WEIGHT AT END OF 36-HOUR FAST	BLOOD SUGAR	GLYCOGEN		URINARY N LAST 24 HOURS	WEIGHT OF ADRENALS (2)	PITUITARY
					LIVER	MUSCLE			
			gm.	mgm. per cent	mgm. per cent	mgm. per cent	mgm./gm. body wt.	gm./100 gm. body wt.	gm./100 gm. body wt.
6	Normal	M	178	68	83	316	0.765	0.0208	0.0039
8	Normal	F	160	70	83	329	0.872	0.0283	0.0053
8	Castrated	M	228	72	124	335	0.878	0.0191	0.0050
4	Castrated	F	194	68	99	356	0.844	0.0191	0.0056
5	Normal 5 day stilbestrol	M	159	76	220	237	0.787	0.0304	0.0057
3	Normal 5 day stilbestrol	F	161	67	282	383	0.686	0.0461	0.0090
3	Castrated 5 day stilbestrol	M	225	79	330	299	0.649	0.0241	0.0050
6	Castrated 5 day stilbestrol	F	196	73	261	347	0.717	0.0340	0.0070
4	Normal 10 day stilbestrol	M	153	76	296	319	0.749	0.0307	0.0064
6	Normal 10 day stilbestrol	F	147	73	354	305	0.791	0.0374	0.0097
3	Castrated 10 day stilbestrol	M	193	77	367	236	0.888	0.0404	0.0085
6	Castrated 10 day stilbestrol	F	191	71	302	421	0.774	0.0415	0.0083
8	Normal 20 day stilbestrol	F	131	84	409	260	1.042	0.0549	0.0110
4	Castrated 20 day stilbestrol	M	205	77	638	387	0.839	0.0388	0.0088
9	Castrated 20 day stilbestrol	F	181	86	577	340	1.018	0.0408	0.0119

received similar treatment. However, a larger number of animals must be studied before this material can be considered as statistically valid.

In animals treated with stilbestrol stored carbohydrates in the form of liver



glycogen were affected most markedly while muscle glycogen showed little variation from the normal. Liver glycogen values for normal fasted rats were a little higher than had been expected (see table 1) and probably reflect the habit of coprophagy which is observed in many rats. In each group of treated animals the liver glycogen was elevated in direct relation to the length of treatment. Although this was a consistent observation within each group, an occasional animal failed to respond to the treatment. These have been included in the averages. The observations of Deuel et al. (6) and Grayman, Nelson and Mirsky (5) on the occurrence of higher levels of liver glycogen in male rats which had received glucose after an initial fast would suggest that we might have expected a sex difference in the glycogen levels of our rats. Although our animals received no carbohydrate after the initiation of fasting, a tendency toward a sex difference in glycogen levels was manifested.

In general, values for liver glycogen were higher in castrated than in normal rats (table 1) and higher in male than in female castrates (table 2). In this regard Zain (7) observed that the administration of Progynon B to hyperthyroid and hyperthyroid-castrated rats resulted in the occurrence of higher values for liver glycogen in the castrates and higher values for males than for females. Our results on normal castrates are similar to Zain's findings on hyperthyroid-castrates. The augmented effect of stilbestrol in castrates may be associated with a lower basal metabolic rate in such animals. Experiments designed to determine this possibility are in progress.

Urine for nitrogen determinations was collected during the last 24 hours of the 36 hour fast. This method is open to some error but it offers an indication of the N.P.N. excretion. As would be expected, the nitrogen values were variable. Since rats which have been treated with stilbestrol lose weight, a rise in the excretion of nitrogen might be expected and it is hazardous to interpret increased N.P.N. levels as being related to such shifts as may have been observed in carbohydrate levels. Nevertheless, females treated for 20 days did show an appreciable rise in urinary nitrogen (table 2) and this may indicate the source of the increased levels of liver glycogen.

Stilbestrol, as is true of the naturally occurring estrogens, has definite effects on many of the endocrine glands. The adrenals and pituitaries were increased in size and, in general, were larger in females than in males. They showed, as a rule, a progressive hypertrophy with continued treatment. No consistent difference was observed in the responses of these glands in normal and castrated rats. The pituitaries of treated animals showed a progressive degranulation of the chromophilic cells and an increase in chromophobic cells.

The ovaries reached a maximum size in rats injected for 5 days and showed large corpora lutea and small follicles. The entire male reproductive system was damaged and the seminiferous tubules and interstitial cells of the testes showed marked atrophy.

The mammary glands of all animals which received stilbestrol showed various degrees of stimulation. In general, the effect was similar to that seen in rats treated with estrone or estradiol (Nelson, 9).

It is evident from these experiments that the action of stilbestrol in increasing carbohydrate levels is not through the gonads. In fact, the liver glycogen values, in general, are greater in castrates than in normals. Further studies are in progress in an attempt to determine whether stilbestrol acts through the adrenals or the pituitary or whether its effect on carbohydrate levels is independent of these glands.

#### CONCLUSIONS

1. Stilbestrol tends to increase blood sugar levels after 20 days' treatment in fasting normal or castrated rats.

2. Liver glycogen values are increased after 5 days of treatment, and considerably more after 20 days. This elevation, in general, is greater in males than in females, greater in castrates than in normals. Muscle glycogen is not altered after the administration of stilbestrol.

3. Urinary nitrogen values are variable but they are somewhat increased after 20 days' treatment, and may indicate the occurrence of gluconeogenesis from protein.

4. The adrenals and pituitaries are increased in size. In general, they are larger in females than in males.

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# OBSERVATIONS ON THE PHYSIOLOGICAL REACTIONS OF THE DUCTUS ARTERIOSUS

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In a previous paper (Kennedy and Clark, 1941), we have pointed out that the ductus arteriosus is a blood vessel having special characteristics which distinguish it from other large vessels, both in its structure and in its physiological reactions. The normal structural characteristics and the changes following normal closure were presented, and it was pointed out that patency of the ductus arteriosus, one of the common forms of congenital heart disease, may be due to the failure of a physiological mechanism rather than to an embryological malformation. The present paper is concerned with physiological reactions of the ductus.

We have already presented evidence to show that the closure of the ductus arteriosus is a process having two distinct phases. The first is an immediate reaction taking place a few minutes after birth and is essentially a contraction of the muscular wall of the ductus (fig. 1). In some species there has been described a flap-like valve (Hamilton, Woodbury and Woods, 1937) which may aid in closure by obstructing the flow of blood from the aorta into the ductus, but this latter mechanism does not appear to be a factor in the guinea-pig fetus, the animal forming the basis of our study (Harmon and Herbertson, 1938). The second phase of closure is much slower than the first and involves histological closure of the lumen and the replacement of the muscular elements in the wall by fibrous connective tissue resulting in the conversion of the ductus into the ligamentum arteriosum.

The ductus seems to have the capacity to close in response to a variety of stimuli, and in this respect its reaction is quite different from that of the aorta and pulmonary artery. We observed that following the onset of breathing in normal guinea pigs closure of the ductus arteriosus occurred within 4 to 10 minutes. After a few experiments it became apparent that closure of the ductus followed artificial inflation of the lungs with air as well as normal breathing. This suggested that the reaction of closure occurred as a reflex response to definite stimuli, and in an effort to throw light on this conception we entered on the present series of experiments.

**MATERIALS AND METHOD.** We have used more than 90 pregnant guinea pigs and made observations on upwards of 175 fetuses. By using the technique described previously, which involves delivering the fetuses by operation beneath

the surface of a warm saline bath and leaving the placental circulation and cord vessels intact, prolonged observations can be made on each fetus and the various reactions of the ductus can be studied. The spinal cords of the mother guinea pigs were sectioned in the lower thoracic region under ether anesthesia in preparation for all experiments except a few and where any of the latter were used in the experiments given as examples, special mention is made of it in the protocols. Most of the fetuses used were near term. In some animals the day of fertiliza-

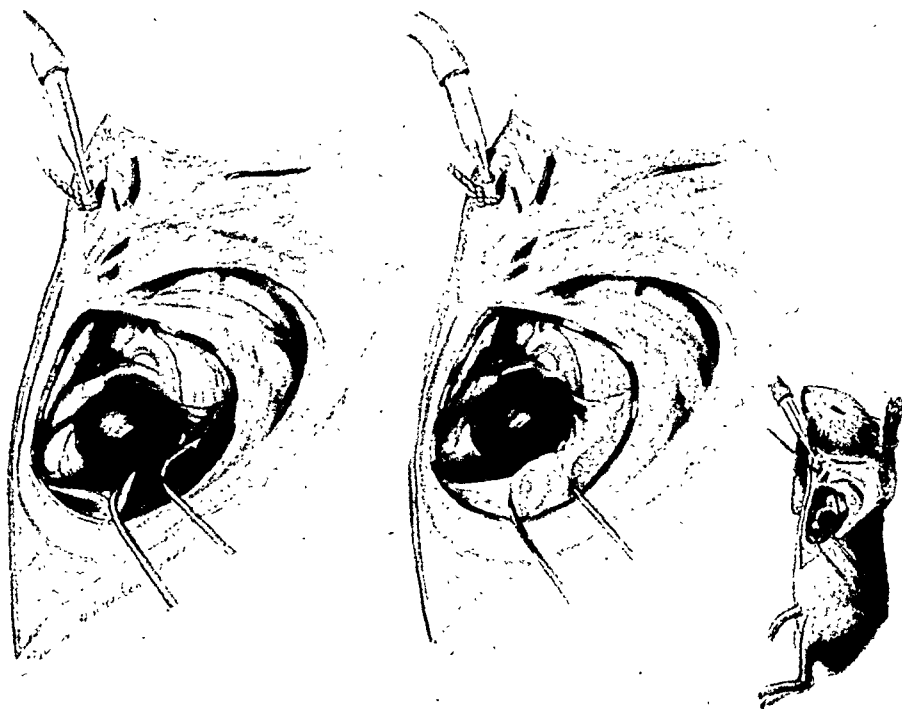


Fig. 1. Drawing showing the appearance of the ductus arteriosus of fetal guinea pig open and closed. In the figure the arch of the aorta is shown in the upper part of the opening of the chest, with the pulmonary artery just below it, apparently continuous (by way of the ductus) with the descending aorta (cf. fig. 2). The ductus in the larger right hand figure closed following artificial inflation of the lungs with air. The lighter color of the lung margin (being retracted below the heart) following inflation is apparent. The smaller figure to the right gives the appearance of the fetus with opening in chest wall. In each figure the tracheal cannula is shown.

tion and length of pregnancy were accurately known. All the fetuses used were weighed and the crown-rump lengths measured. These were compared with the weights and lengths appearing in published data on normal guinea pigs. According to Draper (1920) and Ibsen (1928) guinea pig fetuses at term have an average weight of 80 to 85 grams and are about 10 cm. in length. The individual weights vary with the size of the litter from 70 grams with 6 fetuses to 110 grams with 1 fetus (Ibsen, 1928). The gestation period for guinea pigs has an average duration of 68 days (Ibsen, 1928).

RESULTS. In fetuses near term it was found that the ductus will close following several different types of stimuli (fig. 2) and it will subsequently open again if the stimulus producing the closure is discontinued. This sequence of closing and opening can be repeated at will. For example, intermittent rhythmic inflation of the lungs with puffs of air through a tracheal cannula at a rate of about 50 per minute (to simulate breathing) caused the ductus to close 7 times consecutively in the same animal, each closure being followed by opening, as shown by the following protocol. Protocol 1. Weight of fetus 93 grams; length 11.4 cm. (crown-rump), selected from a group of such experiments.

Fetus removed from uterus 2:08 p.m. Chest opened and ductus arteriosus observed to be open. 2:11 cannula inserted into trachea.

Ductus open.	Times at which successive inflations were begun	Period of inflation required for closure
1.	2:14	2 min., 30 sec.
2.	2:23½	1 min.
3.	2:36½	3 min., 30 sec.
4.	2:47	2 min., 30 sec.
5.	2:56	2 min., 30 sec.
6.	3:05	3 min.
7.	3:14½	2 min., 50 sec.

The ductus arteriosus was allowed to open following each closure.

In lively guinea-pig fetuses near term, this type of inflation of the lungs with air was almost invariably followed by closure of the ductus. It was therefore used as a method for producing closure and various experiments were designed to interrupt the mechanism operating in the hope of determining the underlying causes of closure of the ductus. With the possibility that closure following pulmonary inflation was dependent upon a neurological pathway a series of experiments was performed in which portions of the nervous system were destroyed with the idea of interrupting a reflex mechanism.

*Experimental search for reflex paths.* Subsequent to each of the following procedures the ductus was successfully closed by intermittent inflation of the lungs through a tracheal cannula:

1. Tissue dissected off anterior surface of ductus (including left vagus and phrenic nerves).
2. Bilateral section of vagus nerves.
3. *a.* Bilateral removal of stellate ganglia.  
*b.* Bilateral removal of stellate ganglia and section of both vagus nerves.
4. *a.* Bilateral ligation of carotid arteries below bifurcation.  
*b.* Bilateral removal of carotid arteries including their bifurcation.  
*c.* Bilateral removal of carotid arteries including their bifurcation plus bilateral section of vagus nerves.
5. Ligature pulled very tightly around all vessels, nerves and other structures of mediastinum, cephalic to aortic arch (except trachea).
6. Lesions of spinal cord alone.  
*a.* Cross section of cord at level of 3rd cervical; 6 thoracic; 9th thoracic segments. (Some destruction of cord on each side of section.)  
*b.* Destruction of large portions of spinal cord; from 2nd cervical to 2nd thoracic segment; 3rd cervical to caudal end; 1st thoracic to 11th thoracic segment.

7. Lesions of spinal cord *plus* other structures.

- a. Spinal cord destroyed (T1 to T10) and both vagus nerves cut.
- b. Spinal cord destroyed (C2 to caudal end), all mediastinal structures cephalic to aortic arch (except trachea) tied with ligature and both vagus nerves cut.
- c. Entire spinal cord and medulla destroyed, both stellate ganglia removed, both vagus nerves cut.

*Note:* The procedures listed were carried out on more than one fetus. For example, closure of the ductus following inflation was observed in eight animals after bilateral vagus

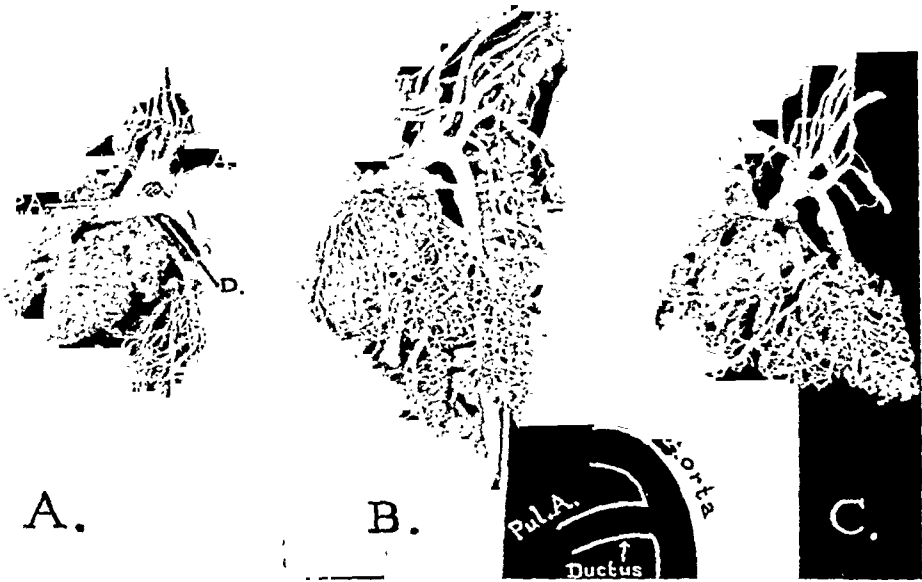


Fig. 2. Photograph of casts of lumina of vessels from injected guinea pig fetuses. The small diagram shows the relation of the great vessels. (A) The ductus arteriosus open. (B) The ductus constricted. (It had closed following inflation of lungs with oxygen but had partly relaxed before the injection fluid was introduced.) (C) The ductus completely closed. This fetus was born normally and injected after several hours of normal breathing. The casts were made by injecting a liquid solution of bakelite<sup>1</sup> into the great vessels of the thorax. After hardening of the bakelite, the tissues were macerated in strong sodium hydroxide solution and washed away leaving a cast of the cavities of vessels injected. (The vertebrae remain in B.) Due to the pressure necessary for injection the recently closed ductus (B) was found to contain a fine thread of the injection mass, which represents the lumen and indicates by its form that the ductus constricted over its entire length. The casts shrink slightly on hardening which accounts for certain irregularities. Magnification  $\times 2$ .

nerve section; in four after bilateral carotid removal; in eight after destruction of the spinal cord; in three after bilateral removal of the stellate ganglion, etc.

The above procedures, in which all known neurological pathways between central nervous system and region of the ductus were interrupted, failed to prevent closure of the ductus following inflation of the lungs with air. The only possible neurological mechanism which might have remained operative would be

<sup>1</sup> Vinylite.

a reflex dependent upon local neurons but there is not supporting evidence for the presence of such a local reflex.

The problem of the mechanism of closure was also approached by a different type of experiment. Various structures were stimulated with an electric current of low voltage (60 cycle sine-wave) and the effect on the ductus noted. We have stimulated in this way the left vagus nerve, right vagus nerve, left cervical sympathetic, the left phrenic nerve, the left stellate ganglion, the left splanchnic nerve without causing any noticeable change in the ductus.

It appears from the above experiments that a nerve pathway or a neuromuscular reflex is not essential for closure of the ductus. It also appears that the ductus will not close following stimulation of certain nerves which are anatomically closely associated with it.

It may be appropriate to point out here that in experiments of this sort there are variables present to disturb the accuracy of the results; such, for example, as interference with the circulation of the fetus, or that of the uterus or placenta, etc. For this reason one positive experiment in which the ductus closed is worth more than one in which no closure occurred. At times when experiments similar to some of those described above as resulting in closure were performed the ductus remained open. Those in which closure occurred are more significant because they show that the destructive lesion did not interrupt the mechanism of closure under the conditions of the experiment.

As previously stated the ductus was observed to close following several types of stimuli other than artificial inflation of the lungs with air. The various procedures employed and their effects upon the ductus can best be shown under the following headings with protocols of a few representative experiments.

1. *Normal breathing.* Protocol 2. Weight of fetus 88 grams; length 11.2 cm. (crown-rump).

Fetus delivered by operation 4:29. Umbilical cord intact. Spontaneous, vigorous breathing began at 4:31. Opening of chest begun 4:35 30". Ductus observed to be closed at 4:35 45".

2. *Mechanical or electrical stimulation of ductus.* Any dissection in the vicinity of the ductus which causes tugging on its wall, or gentle pinching of the ductus with small tissue forceps, or electrical stimulation of its wall is followed by closure. This reaction differs from the preceding one in that closure following a local stimulus occurs more promptly, usually within 15 to 30 seconds, and in that it can occur even a short time after death of the fetus. Similar stimulation of the great arteries of the thorax is not followed by a reaction similar to that of the ductus but the umbilical vessels constrict promptly at the site of mechanical stimulation.

3. *Artificial inflation of the lungs with oxygen and nitrogen.* Protocol 3. The ductus was allowed to open following each closure.

Closure of the ductus with these three methods (normal breathing, direct mechanical or electrical stimulation, and artificial inflation of the lungs) has been observed many times. While artificial inflation failed at times, there was usually observed some factor which might have affected the normal response, such as failure of either the fetal or maternal circulation, length of experiment, temperature of bath, size and age of fetus, etc.

Other observations listed below (sections 4 to 7) have not been repeated a sufficient num-

DUCTUS OPEN. INFLATION OF LUNGS WITH:	NUMBER MINUTES INFLATION	STATE OF DUCTUS ARTERIOSUS AT END OF INFLATION
Fetus 1, weight 128 grams; length 12.3 cm.		
Oxygen.....	6	Closed
Nitrogen.....	10	Open
Oxygen.....	4.5	Closed
Nitrogen.....	7	Open
Oxygen.....	1.75	Closed
Fetus 2, weight 117 grams; length 11.8 cm.		
Nitrogen.....	15	Open
Oxygen.....	6	Closed
Nitrogen.....	8	Open
Oxygen.....	4.5	Closed
Nitrogen.....	5	Open

ber of times to test fully their reliability in closing the ductus but we have observed several valid experiments of each kind.

4. *Injection of adrenalin.* a. Protocol 4. Pregnant female guinea pig decerebrated under ether. Fetus near term (weight and length not recorded). Chest opened. Ductus open. One cubic centimeter of 1/10,000 solution of adrenalin injected slowly into the heart. Ductus closed 3 minutes 10 seconds after injection begun.

b. Protocol 5. Weight of fetus 84 grams; length 11 cm.; 0.5 cc. adrenalin chloride 1/10,000 injected into subcutaneous tissue of abdomen. Ductus closed 2 minutes 20 seconds after injection.

5. *Mechanical stimulation of carotid sinus.* a. Protocol 6. Weight of fetus 72 grams; length 10.2 cm. Left carotid sinus massaged with blunt end of probe. Ductus which had been open, closed after 2 minutes' massage.

b. Protocol 7. Weight of fetus 71 grams; length 10.2 cm. Right carotid sinus massaged with blunt end of probe. Ductus which had been open, closed after 4 minutes 20 seconds' massage.

6. *Hemorrhage.* Protocol 8. Weight of fetus 70 grams; length 10 cm. Ductus had remained open and under observation for 25 minutes. Left external jugular vein was cut for purpose of causing hemorrhage which was short and brisk. Ductus closed 6 minutes after beginning of hemorrhage.

7. *Unexplained.* On several occasions we observed closure of the ductus and were not able to relate it definitely to any of the above factors. For example, in about half a dozen experiments after the tracheal cannula was inserted the ductus closed; or after destruction of the spinal cord by thrusting a pipe cleaner into the vertebral canal; or after opening the chest wall with an associated small hemorrhage the ductus closed; or after the position of the fetus was changed suddenly, and occasionally after the mother struggled, the ductus closed. On the whole these unexplained closures were infrequent but they suggest that there may be factors operating to cause closure which we do not understand and do not at present recognize. It may well be that closure which followed some other procedure should be grouped here, or that some of these experiments belong under another heading.

8. *Intravenous injection of oxygen.* From the evidence that has been presented it is difficult to arrive at a conception of the cause of closure of the ductus which will fit all the facts observed. The most promising observations from the standpoint of a mechanism used at birth are those concerning the different reactions following inflation of the lungs with air, oxygen and nitrogen. According to our experiments, inflation of the lungs with



pure nitrogen does not result in closure of the ductus and it seems that oxygen is an important constituent of the gas mixture when closure follows inflation of the lungs. If oxygenation of the fetal blood causes closure, then oxygen given to the fetus by any route resulting in oxygenation of the blood should have the same effect. In order to test this hypothesis the following experiment was devised. With the usual technique the ductus was visualized and by means of a small needle and a tuberculin syringe pure oxygen was slowly injected into the umbilical vein in a series of tiny bubbles. This procedure was promptly followed by closure of the ductus in four animals. In one other, whose heart was beating irregularly a partial constriction of the ductus occurred. In two others the technique of injection failed and the experiment could not be completed. In no one of these animals were the lungs inflated before the oxygen was injected.

Protocol 9. Weight of fetus 69 grams; length 11.3 cm. Oxygen injected into umbilical vein very slowly with small needle. The ductus which had been open, closed after 3 minutes 15 seconds, a total of 0.3 cc. of oxygen was injected.

*Note:* The irritability of the umbilical vessels to needle puncture can be abolished by first painting the outside of the cord with formalin (10 per cent), then injecting a few tenths of a cubic centimeter of formalin into the mucous connective tissue of the cord around the vessels.

**DISCUSSION.** We have established that the ductus arteriosus is a structure which can actively close in response to certain stimuli. It responds to local mechanical stimulation much the same as certain other hollow muscular structures by contracting. We do not believe that local mechanical stimulation has an essential rôle in its closure under physiological conditions. Neither does a neurological mechanism appear essential to closure following artificial inflation of the lungs. Our findings are at variance with those of Barcroft, Kennedy and Mason (1938) with respect to the reaction of the ductus following stimulation of the vagus nerve, but we believe that the present observations have been adequately controlled.

Of the stimuli causing closure of the ductus which we have explored, it seems likely that under physiological conditions, breathing is the most important. The actual filling of the lungs by just any gas is not sufficient. From our experiments it appears that oxygen is a necessary component of the gas mixture since inflation of the lungs with pure nitrogen will not cause closure. Oxygen by vein will also cause closure without the necessity of accompanying inflation of the lungs. It is quite possible that many or all of the unexplained closures (see sec. 7) could be due to an increased oxygenation of the fetal blood in response to painful stimulation, struggling of the mother or fetus, hemorrhage, etc. There are other possible sources of stimulation which we have not yet explored fully, such as various natural humoral substances,  $\text{CO}_2$ , drugs, etc.

Such an influence as that of oxygen on the ductus may have something in common with the findings of Figge (1934) who demonstrated a definite effect on the metamorphosis of the aortic arches and gills in larval forms of the salamander by variations in oxygen tension of their environment.

If this seemingly important relationship of oxygen to the mechanism of closure of the ductus is true, it offers a practical indication for treatment of new-born infants, especially those which have difficulty in the oxygenation of their blood.

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# TRAINING AND ITS EFFECTS ON MAN AT REST AND AT WORK

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In exhausting activity, such as in competitive sports and in war, the vigor and extent of exercise are circumscribed by the limits of the physiological functions involved. We know that at a given age and under fixed conditions, the heart rate cannot be pushed beyond a certain value. At any given time the capacity for supplying oxygen to the tissues is strictly limited. The individual will work anaerobically until a tolerable limit of oxygen debt and concentration of lactic acid is reached. Severe prolonged work may be limited by the stores of carbohydrate.

Yet it is a common observation that exercise repeatedly carried out leads to an improved performance. In the runner this amounts to running a greater distance at the same pace, or covering the same distance more quickly, or covering the same distance at the same rate with less fatigue. As soon as improvement in performance can be demonstrated, the process of training may be said to have begun. The rate of improvement depends on the individual's initial state and on how rigorous a regime he follows. Any regime systematically followed will have its most striking results following a few weeks of training; after the first rapid gains, hard diligent work is required if a continued improvement is to be secured. Such training is essential for success in amateur or professional sport. The less seriously one takes his sport, the less impressive is the second phase of improvement: the average young man can soon acquire the ability to run a mile in six minutes but he must work hard and long to run it in five minutes.

The means by which training results in an increased capacity for work is not completely understood. Experimental work done on animals and their musculature, and on intact humans, has yielded some significant advances, but some of the evidence is conflicting. An excellent review of work on training was made by Steinhaus (1933), and later Dill (1936) reviewed various aspects of muscular exercise. More recent summaries of the literature related to the problem are those of Hellebrandt (1940), Steinhaus (1941) and the closely related studies of muscular contraction as reviewed by Sacks (1941). Extensive discussions are found in Bainbridge (1931) and Schneider (1939). Many of the studies on training have compared two selected populations, one, a group of trained men and the other, a control group. This type of study yields less useful data than one in which individuals are studied before and during training.

Two years ago simultaneous studies of training were begun in the Fatigue Laboratory and in the Department of Physiology of the University of Indiana.

The subjects consisted chiefly of non-athletic students. The plans for laboratory performance tests and observations were similar but separate paths were followed in order to obtain independent judgments as to the outcome of the experiments. Some of the Indiana results have been published by Robinson and Harmon (1941), and the review in their paper of recent literature is so adequate that it need not be repeated here. It is now clear that the Indiana students showed greater improvement and reached higher levels of performance than did the Harvard students. These differences probably depend on a number of factors, some of them psychological. The students at Indiana were working their way through college and were paid for their time in these tests. Those at Harvard were fulfilling the requirement that all Freshmen take part in some physical activity. They too were paid but not all of them were dependent on this source of income. While both groups had track work three times weekly there is no comparative record of the amount of training received nor of the degree to which individuals pushed themselves during training. The Indiana student, in the words of Doctor Robinson, "were given about as much running as they could take but this was considerably short of what is expected of a veteran runner. The amount of running done by a beginner is limited by shin-splints and arch trouble. These may occur even in some veterans if too much work is attempted."<sup>1</sup>

The observations made in our laboratory consisted of bimonthly studies of the men at rest and biweekly studies of work performance. We measured basal metabolic rate, analyzed alveolar air, recorded breathing curves and vital capacity, obtained resting pulse and blood pressure values. Venous blood drawn during each bimonthly test was analyzed for O<sub>2</sub> capacity, alkaline reserve, plasma protein and chloride. A study was made of the formed elements of the blood and hemoglobin concentration. At the same intervals, the men collected three 24-hour urines for study of possible effects of training on excretion of chloride, creatine and creatinine.<sup>2</sup>

The progress of the subjects was followed biweekly by means of work experiments on our motor-driven treadmill. The subject walked eight minutes at 3.5 m.p.h. on an 8.6 per cent grade, and then immediately ran on the same or a higher grade at 7.0 m.p.h. for five minutes if possible. Otherwise he stopped when exhausted. At the end of the run the subject jumped to the side or straddled the belt, keeping the mouthpiece in place. As soon as the belt stopped a stool was placed on it. The subject sat on the stool during a 15-minute recovery period. Oxygen consumption was measured for the last part of the walk, for each minute of the run and for the recovery period. A continuous heart rate record was made of the entire experiment. Capillary blood was taken after the

<sup>1</sup> Personal communication.

<sup>2</sup> Nearly all of our subjects ate at the dining hall provided for Freshmen and hence had the same choice of food. There was no control exercised over their diet except that over a six-week period some time during the six months each man took 60 grams of gelatin daily. There was no evidence that the gelatin influenced performance nor the training curve, that is, the rate at which performance improved.

run for the determination of sugar and lactate. Since the treadmill grade was adjusted up and down during the series of experiments, the subjects were not exhausted at the end of five minutes in some instances. The experiments reported here, however, are those in which the subjects became exhausted within five minutes. These series were at approximately the same intervals as the basal tests.

**RESULTS.** *Basal state.* The mean results of the experiments on fasting and resting subjects are shown in table 1. The first figures represent the initial

TABLE 1

*Summary of the means of data on the basal state of fourteen subjects in training for middle-distance running over a period of six months*

	CONTROL	TRAINING PERIOD		
		After two months	After four months	After six months
Height, cm.....	176.1	176.1	176.1	176.1
Weight, kgm.....	69.6	70.8	72.0	71.8
Heart rate.....	66.8	62.8	63.2	61.8
Blood pressure, mm. Hg.....	114/65	113/65	111/63	113/67
Respiratory rate.....	15.1	14.6	14.1	14.3
Respiratory vol., l./min. NTP.....	6.23	5.73	5.60	5.69
Vital capacity, l.....	3.79	3.67	3.70	3.63
Basal metabolism, Cal./m. <sup>2</sup> /hr.....	41.5	40.6	40.8	40.9
Alveolar pCO <sub>2</sub> , mm. Hg.....	41.5	40.8	40.6	41.3
Alveolar pO <sub>2</sub> , mm. Hg.....	100.3	104.4	100.8	98.1
O <sub>2</sub> Capacity, vols. %.....	19.83	20.24	20.14	20.18
Alkali reserve*.....	47.8	46.9	47.6	46.6
Plasma chloride, m.Eq./l.....	103.6	104.8	106.0	105.8
Plasma nitrogen, gm./l.....	10.3	10.7	10.7	10.8
Hemoglobin, gm./100 ml.....	14.8	15.1	15.0	15.2
Red cells, millions/mm. <sup>3</sup> .....	4.62	4.82	4.72	4.73
White cells, per mm. <sup>3</sup> .....	5960	6120	5780	6090
Hematocrit, %.....	44.1	44.7	45.2	46.0
Urine chloride, m.Eq./day.....	186	210	181	201
Urine total creatinine, gm./day.....	1.85	1.92	1.84	1.81

\* Defined as the CO<sub>2</sub> combining capacity of oxygenated blood, measured at 37°C. and a pCO<sub>2</sub> of 40 mm. Hg.

values before training began. The next three were obtained after approximately two, four, and six months of training.

Most of the measures in rest, which have been previously thought to be related to the degree of training, appear to remain relatively constant. For example, the alkaline reserve and the alveolar CO<sub>2</sub> tension remain practically level, and if a tendency must be noted, it is for a decrease, rather than an increase, as found in numerous previous studies. On the other hand, there is a decrease in the resting pulse rate of five beats per minute, which fits nicely into the generally accepted picture of training. The gain in weight, amounting to

slightly more than 2 kgm., may be related in whole or in part to the training regime.

Blood pressure, respiratory rate, vital capacity and the basal metabolism were not significantly altered by training. Oxygen capacity of venous blood rose by about 2 per cent, an insignificant change. The increase in concentration of the plasma chloride was at the outside less than 3 per cent, but the change was so consistent that the increases by the fourth and sixth months of training were statistically reliable.

The remaining data on hemoglobin, the formed elements of the blood and the excretion of chloride, creatine and creatinine were more variable in the subjects from test to test, and showed no significant relation to the regime of training.

*Grade-walking.* In the walk, which was always at the same grade and rate, experienced subjects reach a steady state within three or four minutes of starting, carrying on aerobically without a mounting oxygen debt. The oxygen requirement for this grade of work showed the greatest decline within two months, with a further slight decrease at the end of six months of training (table 2). This is reflected in an increased net efficiency at this rate of work of about one-tenth.

TABLE 2  
*The mean changes in the efficiency of grade walking*

	O <sub>2</sub> PER MINUTE		NET EFFICIENCY		HEART RATE	
	l./min.	Δ%	Per cent	Δ%	Beats/min.	Δ%
Control.....	1.91		15.3		151	
After 2 months.....	1.81	-5.2	16.6	+8.5	145	-4.0
After 4 months.....	1.81	-5.2	16.8	+9.8	145	-4.0
After 6 months.....	1.78	-6.8	16.9	+10.4	146	-3.3

These two functions are not exactly mirrored because the measurement of oxygen requirement has not been adjusted by subtracting the basal O<sub>2</sub> requirement nor has it been related to body weight. These points are taken into account in measuring efficiency. The increased efficiency of grade walking was accompanied by a decreased heart rate of about 4 per cent. The mean values of R.Q. for the four series were 0.94, 0.93, 0.92, 0.93 respectively, reflecting an extraordinary constancy in the proportion of carbohydrate utilized.

The improved performance in the walk probably depends on better skill and coördination. It is not likely that the training program had much to do with this improvement for even skilled runners may be clumsy and inefficient in their first walk on the treadmill.

*Maximal work.* The experiments calling for the exhaustion of the subject within five minutes necessitated an increase in grade as the training progressed. The mean grade was increased from 9.1 per cent in the control series to 10.9 in the second, to 13.3 in the third, and to 13.4 per cent in the last series. Observations made on one of the men after he had attained a high capacity are shown in table 3. The mean duration of run for these same series was 3.22, 3.87, 3.17 and

3.44 minutes respectively. Thus, in the last test the run was 7 per cent longer, while the grade was almost one-half greater than in the first test. When the body weight is taken into account, the duration and rate of work yield the total physical work done.

It is of great importance to recognize that the principal increase in work output was accomplished by the increase in rate of work, the duration of work not varying greatly. Under these conditions gains in rate of work performance must decline approaching an asymptote, however rigorous the training. On the other hand, if the rate of work output is kept constant, there may be an enormous increase in the quantity of work that can be done. Thus, with the rate constant, Karpovich and Pestrecov (1941) had a subject who was exhausted after 12 minutes work at the beginning of their tests, but who was able to work for 5 hours, 16 minutes several weeks later. This represents a gain of over 2500 per cent in total work output.

TABLE 3

*Typical data on the performance of one man (J. Y.) in exhausting work*

		HEART RATE IN RECOVERY
<i>Work</i>		
Treadmill speed	(11.3 km.p.h.).....	At 20 sec., 187 beats/min.
Treadmill grade	(15.8%).....	At 40 sec., 178 beats/min.
Duration of run	(3.63 min.).....	At 60 sec., 171 beats/min.
Physical work	(7000 kgm.-m.).....	At 90 sec., 158 beats/min.
Maximal oxygen transport	(3.53 l./min.).....	At 2 min., 147 beats/min.
Maximal heart rate	(197 beats/min.).....	At 3 min., 132 beats/min.
		At 4 min., 124 beats/min.
		At 5 min., 123 beats/min.
<i>Recovery</i>		
Net O <sub>2</sub> debt (15 minutes)	(8.63 l.).....	At 10 min., 112 beats/min.
Maximal lactate	(144 mgm. %).....	At 15 min., 113 beats/min.
Maximal blood sugar	(163 mgm. %).....	

To translate the accomplishment of our subjects into more familiar units, we may say that our average man, while running up hill at seven miles per hour could raise his body 180 feet in 3.22 minutes at the beginning, and 284 feet in 3.44 minutes six months later.

Exhausting experiments are characterized by a mounting oxygen debt, indicating that the body processes are working beyond a point where equilibrium can be attained, and much of the work is carried on anaerobically. The mean increase of about 60 per cent in work done through the training period was in part due to a greater use of the anaerobic mechanisms for energy transformation (table 4). This is indicated both by the increased tolerance for lactate of as much as 18 per cent, which is paralleled by a smaller increase of 5 or 6 per cent in the oxygen debt as judged by the amount repaid in the first 15 minutes of recovery. There was also an increase of about 6 or 7 per cent in the transport of oxygen to the tissues during work, which represents a clear-cut gain in the amount of work which could be carried on aerobically. The amount of oxygen

debt measured during the first 15 minutes of recovery from work of this nature represents a proportion of the total debt that may vary from one-half to two-thirds. The index to anaerobic work so obtained is not precise but even making the most unfavorable assumptions it is clear that only a small fraction of the increased work output can have been accomplished by increased anaerobic energy transformation: it must be attributed to improved efficiency in running.

It is interesting to note that the concentration of lactate reached increased about 10 per cent in the second series relative to unit oxygen debt as measured for fifteen minutes. The ratio stayed virtually constant for the remainder of the experiment. The figures were 20.4, 22.5, 22.4 and 22.6 mgm. per cent lactate per liter of oxygen debt. The constancy of the figure leads one to believe that this change was real, whatever its basis.

An analysis of the heart rate data in recovery after maximal work showed that not only was the mean maximal heart rate at the end of the run approximately

TABLE 4

*The mean changes in the capacity for exhausting work during training*

	WORK DONE TO EXHAUSTION		MAXIMAL LACTATE		MAXIMAL BLOOD SUGAR	
	Kgm.-m.	Δ %	Mgm. %	Δ %	Mgm. %	Δ %
Control.....	3786		114		127	
After 2 months.....	5593	+47.7	131	+14.9	142	+11.8
After 4 months.....	5573	+47.2	135	+18.4	134	+5.5
After 6 months.....	6046	+59.6	134	+17.5	134	+5.5
	WORK PER LITER O <sub>2</sub> DEBT		MAXIMAL O <sub>2</sub> PER MINUTE		O <sub>2</sub> DEBT FOR 15 MINUTES	
	Kgm.-m.	Δ %	l.	Δ %	l.	Δ %
Control.....	484		3.45		7.82	
After 2 months.....	691	+42.7	3.64	+5.5	8.09	+3.4
After 4 months.....	671	+38.7	3.69	+7.0	8.30	+6.2
After 6 months.....	737	+52.2	3.69	+7.0	8.20	+4.9

constant but neither did the regime of training materially alter the course of the recovery heart rate. The data are charted in figure 1. The curves appear to be exponential, and can be fitted reasonably well from the first to the fifteenth minute of recovery. The general form of the curve can be expressed, using the exponential function, as  $y = ae^{-bx} + c$  where  $y$  = heart rate and  $x$  = the time elapsed. The curves have been calculated but do not yield any more information than can be obtained from the chart shown. The important thing we wanted to know was whether the heart rate declined more rapidly or reached a lower level as a result of training. Obviously, the curves under the stated conditions are virtually unaffected by training.

DISCUSSION. In the exhausting grade of work we found that the capacity for aerobic energy transformation had been increased, as evidenced by the increase in maximal oxygen intake. This probably depends on an increased cardiac output, on improved circulation to the working muscles, and on more



favorable conditions for exchange of gases between the capillaries and muscle cells.

The increased level of lactate noted in our subjects may be looked upon as one of the most significant effects of training. We have been accustomed to say that the level of lactate at the end of work is a measure of the extent to which a man has pushed himself. It has been recognized that at best this is a rough approximation. In high altitudes Edwards (1936) has shown that the

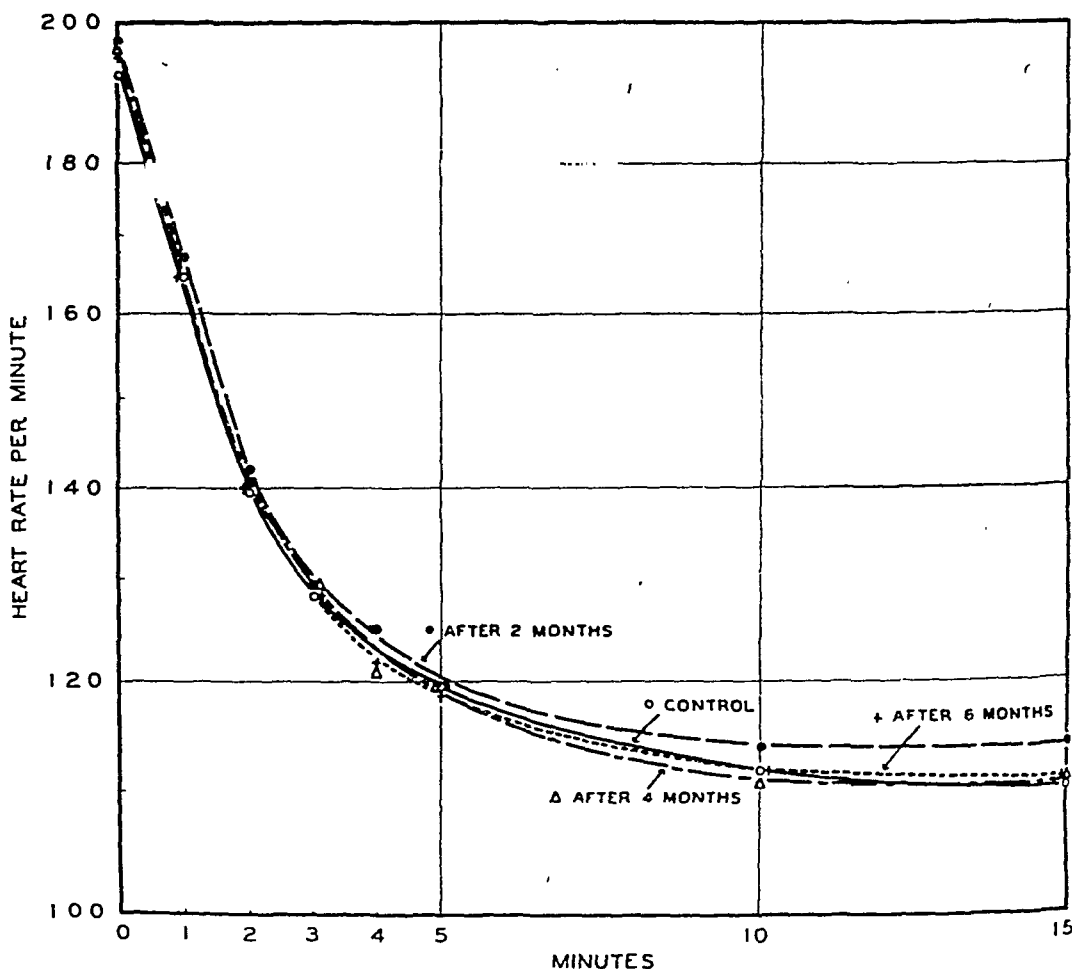


Fig. 1. Training and the heart rate decrement following exhausting work on the treadmill.

capacity of an individual to accumulate lactate in exhausting work falls off as the degree of anoxia, or the altitude, increases. Robinson (1938) has shown that old men, even though healthy, cannot accumulate much lactate: the capacity for anaerobic work falls off with increasing age. On the other hand, the highest lactates we have seen occur in athletic young men. The ordinary young man will stop work with a lactate of 100 mgm. per cent while first-class athletes will continue until their lactate level has reached 150, 175 or even 200 mgm. per cent as has been shown by Robinson and Harmon (1941).

We know that in high altitudes despite the inability to reach sustained high levels of performance in climbing, the individual may have unimpaired muscle strength. Furthermore we have seen excellent performances in strength tests in men past middle age who were incapable of reaching a high level of lactate in work that taxed the capacity for supplying oxygen. These observations suggest that the capacity to accumulate lactate runs parallel and may in fact furnish an excellent index to cardiovascular fitness.

Increases in oxygen intake and in oxygen debt account only in part for the increase during training in the rate at which external work can be done. The useful work output increases in relation to the oxygen requirement.

One of our negative findings is interesting in the light of current indices of physical fitness. Many of these use the decline of pulse rate after performance of a fixed task as one of a battery of tests of physical condition of a subject. We find that a regime of training, which certainly increases physical fitness, does not alter the decline of heart rate following exercise to complete exhaustion within an approximately constant time limit of three to four minutes. A study of the decrement in heart rate after exercise of fixed intensity and duration would certainly show a more rapid pulse recovery during any effective period of training. Our data do not negate such findings nor do they run counter to the assumption that individual differences in performance can be related to decline of pulse rate after moderate exercise. They do seem to show that when complete exhaustion is reached in a given time, the rate of work varying, the pulse recovery curve remains unaffected by training. This is in harmony with the findings of Robinson and Harmon (unpublished data—personal communication).

#### SUMMARY

A group of fourteen subjects followed a training regime for middle-distance running over a period of six months. The men were studied before and during this period at rest and while doing two grades of work on a motor-driven treadmill.

The training regime was accompanied by a slight increase in weight, a decrease in resting pulse rate of five beats per minute, a slight decline in the respiratory rate and volume and a slight increase in plasma chloride.

No significant differences were found in alveolar  $\text{CO}_2$ , alkaline reserve, metabolic rate, hemoglobin or the formed elements of the blood, all being measured in the resting state.

An increase in efficiency of grade walking was observed.

In exhausting work there was an increased capacity for supplying oxygen to tissues and greater utilization of anaerobic energy reserves. The work increment unaccounted for by these alterations presumably results from a more economical organization of bodily functions.

The increased capacity for accumulating lactic acid that is developed during training, and the notably high lactate levels attained by first class athletes points to this determination as a useful index to cardiovascular fitness.

We are indebted to Dr. Clark W. Heath for hematological studies reported here and to Mr. Frank Consolazio for invaluable technical assistance.

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# THE REACTION AND NEUTRALIZING ABILITY OF THE CONTENTS OF THE PYLORIC ANTRUM AND FIRST PART OF THE DUODENUM IN NORMAL DOGS FOLLOWING AN EWALD MEAL<sup>1</sup>

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Acid has been accorded a leading rôle in both the pathogenesis (2, 3, 9, 10, 11) and the maintenance (3, 6, 9, 10, 11, 12) of peptic ulcer. This study was undertaken primarily for the purpose of learning more about the acidity of the contents of the pyloric antrum and the first part of the duodenum in normal animals. It is hoped that the knowledge gained in this and subsequent investigations will shed some light on the pathologic physiology of peptic ulcer. We hope also to learn whether gastric and duodenal acidities vary independently or follow a parallel course. Such knowledge should assist in evaluating current diagnostic and therapeutic measures which are directed almost exclusively toward measurement and modification of gastric acidity in spite of the fact that at least 85 per cent of the ulcers encountered clinically are in the first part of the duodenum (3, 4, 7, 8, 13).

The observations of duodenal acidity will also extend our knowledge regarding the availability of acid in the intestinal contents for the regulation of gastro-intestinal functions such as gastric and pyloric motility and pancreatic and gastric secretion.

**METHOD.** Dogs averaging about 20 kgm. in weight were prepared with cannulated gastric and duodenal fistulas as described in previous reports from this laboratory (16, 17). No observations were made for 3 to 4 weeks following operation. All the animals were trained to stand quietly in a muslin hammock-support before any experiments were attempted. The basal routine diet between the experimental periods consisted of daily feedings of raw beef for 5 to 6 days followed by "Purina" dog chow for 1 to 2 days.

<sup>1</sup> Portion of thesis submitted by Dr. Berk to the Faculty of the Graduate School of Medicine of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Medical Science (D.Sc. (Med.)) for graduate work in internal medicine.

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After a fasting period of 24 to 36 hours, sampling tubes were introduced through the gastric and duodenal fistulas consisting of 2 soft rubber tubes perforated for a distance of 1 inch from their tips, which were joined together by a thread  $\frac{3}{4}$  inch long (fig. 1). The tubes were so placed that the thread came to lie within the pylorus, thereby permitting specimens to be collected from areas 1 inch long on each side of the pylorus. In addition, a small soft rubber instillation tube perforated near its tip was introduced through the gastric fistula (fig. 1). A diagrammatic schema of the final arrangement is shown in figure 2.

After a rest interval of  $\frac{1}{2}$  hour, samples in the fasting state were taken simultaneously from the gastric and duodenal segments at 10 minute intervals for another  $\frac{1}{2}$  hour. At the completion of this period the dogs were fed an Ewald meal consisting of 2 pieces of stale bread or toast and

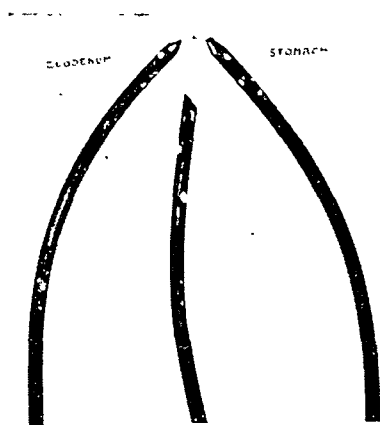


Fig. 1

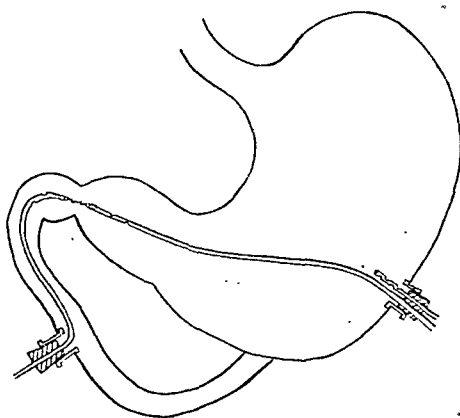


Fig. 2

Fig. 1. Sampling and instillation tubes used in cannulated fistulas.

Fig. 2. Diagram of stomach and duodenum showing the approximate location of the cannulated fistulas and the manner of arranging the sampling and instillation tubes.

250 cc. of tap water at room temperature. The water was introduced through the gastric instillation tube because of the refusal of the dogs to drink it. Beginning 10 minutes after the start of the meal, samples were simultaneously removed from the gastric and duodenal segments at 10 minute intervals for  $2\frac{1}{2}$  hours and at  $\frac{1}{2}$  hour intervals for another hour. In a few experiments samples were removed at other times in addition to those stated.

The pH of each sample was determined electrometrically through the use of a Leeds-Northrup pH indicator, which uses a glass electrode, and the titratable free acid and total acidity were measured, using dimethyl-aminoazobenzene (Toepfer's reagent) and phenolphthalein as the respective indicators. On each duodenal specimen, in addition, there was determined what was called the excess neutralizing ability. This indicated the amount of hydrochloric acid (N/10) which could be introduced per 100 cc.

of duodenal contents before the reaction for free acid with Toepfer's reagent became positive. In expressing the results the 4 specimens collected in the fasting state were averaged to obtain a single fasting value.

Twenty-seven acceptable experiments were performed on 6 dogs. All experiments which were done on animals who were later found to have been ill or who vomited and those in which there was uncertainty as to the duration of the fasting state or the position of the tubes were discarded. Of the acceptable experiments, 20 were complete in that they were carried out for the standardized period, and 7 were incomplete in that they were carried out for periods shorter than the arbitrarily selected standard. In all, 2726 different observations were made consisting of 801 determinations of pH, 786 measurements of free acid, 764 measurements of total acidity and 375 measurements of duodenal excess neutralizing ability.

**RESULTS.** *pH* (fig. 3). There was a consistent difference in pH of samples collected simultaneously from just above and below the pylorus. In both the fasting and post-meal phases, this difference averaged about 3 pH units. The pH values of the contents of the first part of the duodenum displayed a wider range than did the contents of the pyloric antrum. Both, however, failed to follow any regular type of curve, tending rather to fall on a flat although irregular line.

*Free acid* (fig. 4). The average free acid found in the stomach in the fasting state is probably high, especially for dogs. The foreign body effect of the tubes may have contributed to the production of the high acidity so we do not regard it as especially significant. Following an initial decrease in free acid immediately after the meal, due presumably to dilution effect, there was a progressive increase which tended to stabilize at the peak level at the termination of the period of observation. The increase in titratable gastric free acid was in striking contrast to the absence of any corresponding decrease in gastric pH (fig. 3).

With our technic the contents of the first part of the duodenum characteristically showed an absence of free acid. Out of 410 determinations of free acid in both the fasting and post-meal phases only 11 were positive for free acid (2 fasting and 9 post-meal) and the highest value obtained was 16 clinical units. Our method of preparation of the sample for titration purposes consisted of adding 10 cc. of distilled water to 1 cc. of the strained contents. The pH readings were made on the unfiltered, undiluted specimens as obtained from the sampling tube. The pH at our free acid (Toepfer's reagent) end point averaged 3.36 with a range of 2.88 to 3.72 (1). It will be seen in figure 3 that in the post-meal phase alone 56 of the 342 determinations of pH in the unfiltered, undiluted specimens were 3.5 or below. All of these would be expected to show a positive reaction for free acid on the basis of the pH at our end point with Toepfer's reagent. Yet, after filtration and dilution only 9 or 2.6 per cent yielded positive reactions for free acid. We were able to demonstrate that this error was due primarily to the effect of dilution (1). The average in-

crease in pH effected by dilution was 0.6 unit. Even after qualifying our figures to allow for this error, it may be said that the immediate admixture

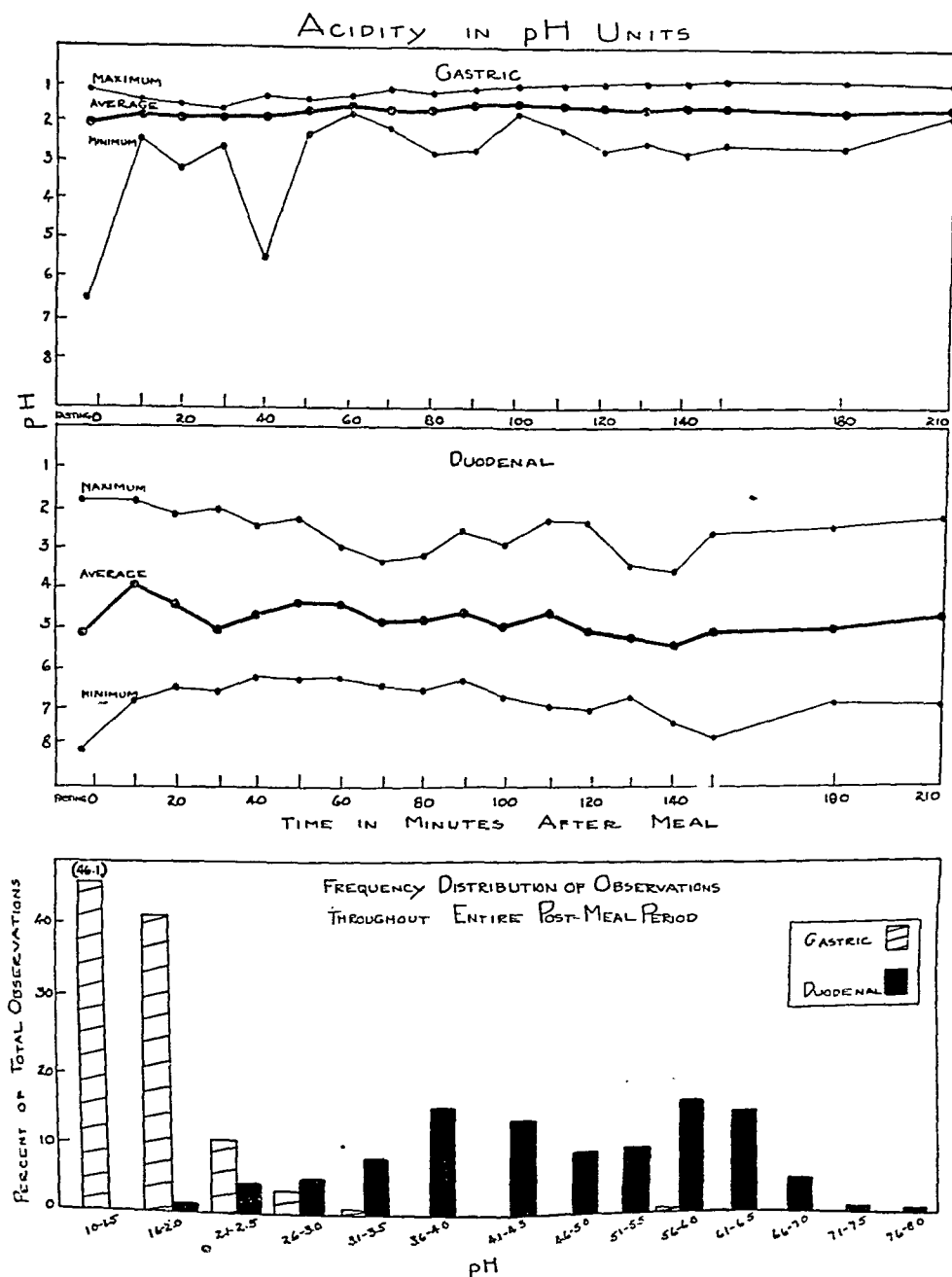


Fig. 3. Acidity in pH units of samples collected simultaneously from just above and just below the pylorus.

of the gastric chyme and the contents of the first part of the duodenum in normal dogs following an Ewald meal will result in complete neutralization of the free acid in 80 to 85 per cent of the samples.

Total acidity (fig. 5). Essentially the same remarks apply to total acidity within the stomach as were made in regard to free acid. A rough

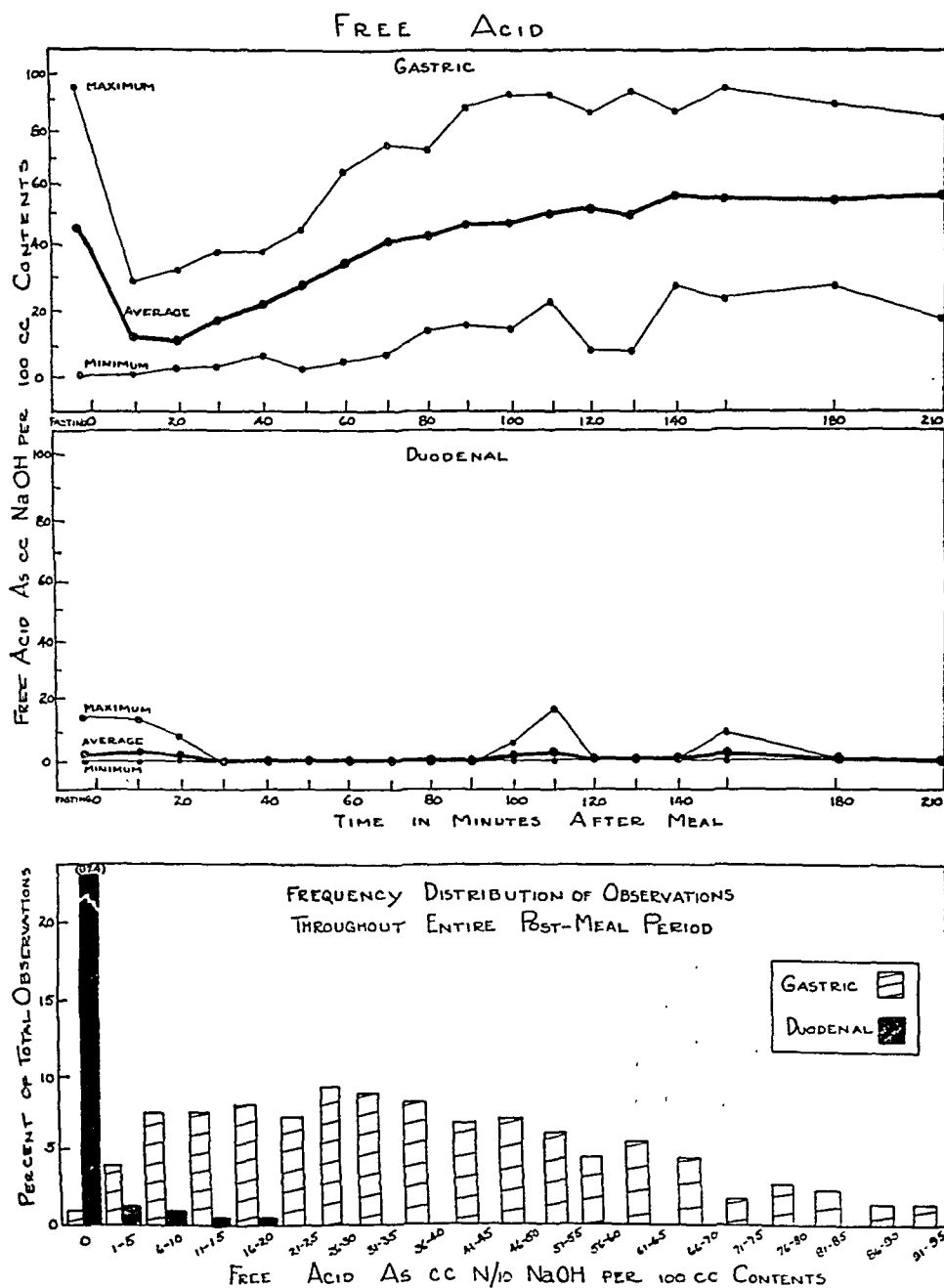


Fig. 4. Free acid as determined on samples collected simultaneously from just above and just below the pylorus.

curve of total acidity can be made out in the duodenal bulb which has a similar configuration to, although it is widely separated from, that seen in the stomach. In both the pyloric antrum and the first part of the



duodenum the increase in total acidity is again in contrast with the lack of any corresponding decrease in pH in either region (fig. 3).

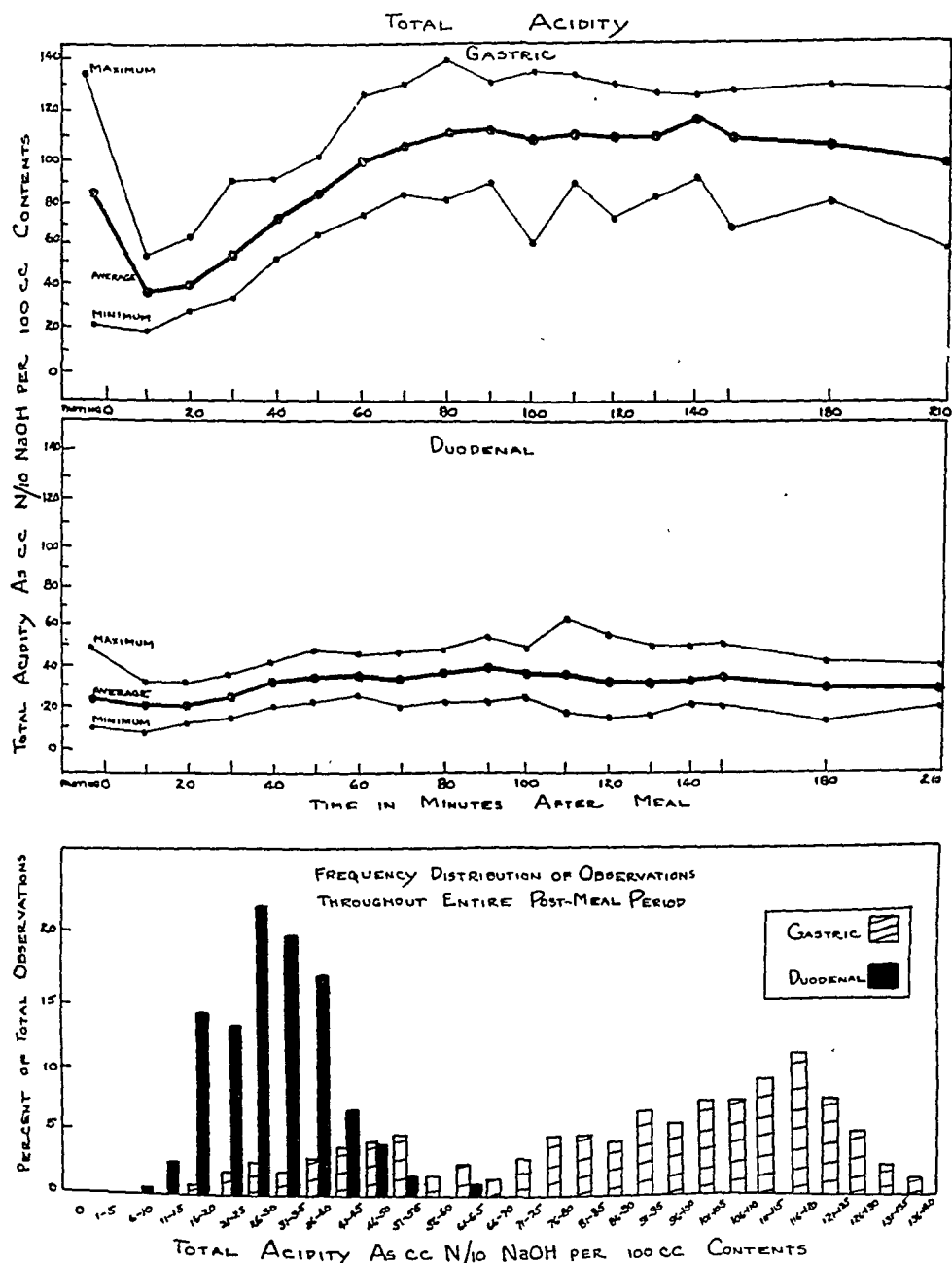


Fig. 5. Total acidity of samples collected simultaneously from just above and just below the pylorus.

*Excess neutralizing ability of the duodenal contents* (fig. 6). It was noted above (fig. 4) that when gastric chyme and duodenal contents were admixed in the duodenal bulb there occurred almost immediate neutral-

ization of the free acid in the vast majority of post-meal specimens. In order to determine how well the contents of the first part of the duodenum were equipped to neutralize the acid received from the stomach, its excess neutralizing ability was measured. This was done by adding N/10 hydrochloric acid to 1 cc. of the strained, admixed duodenal contents diluted with 10 cc. of distilled water to which 4 drops of Toepfer's reagent was added. The end point was the same as that used for the titration of free acid.

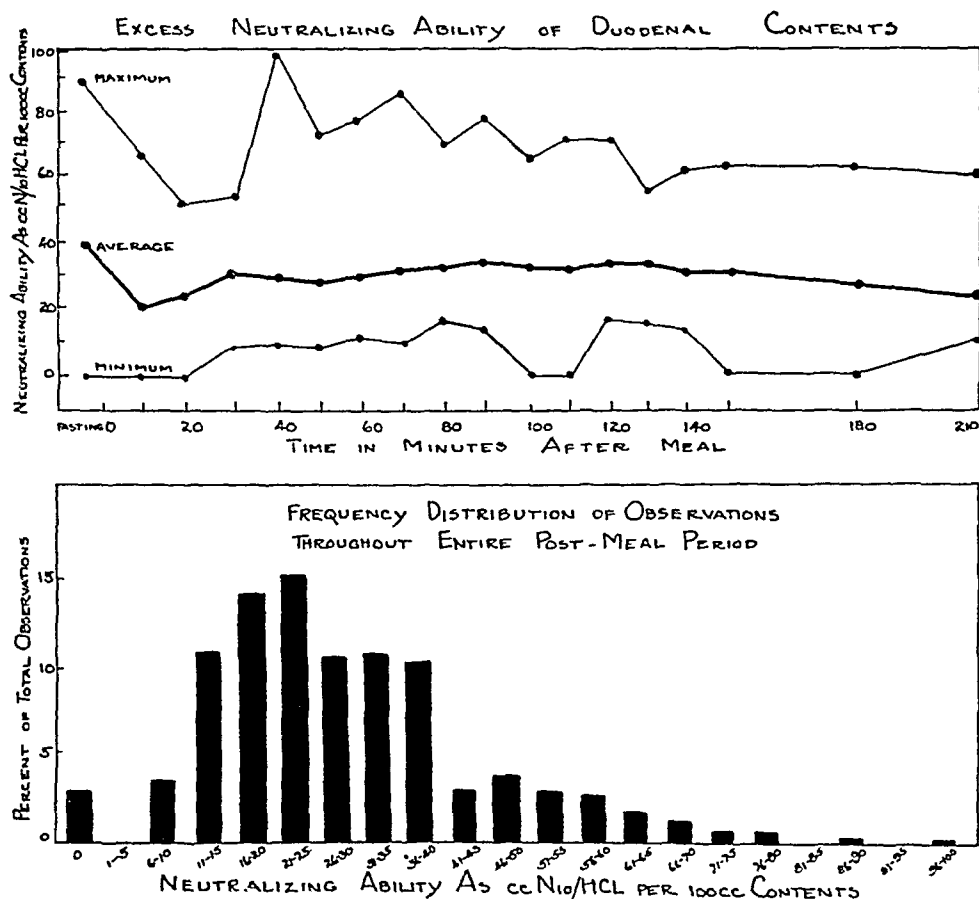


Fig. 6. Excess neutralizing ability of the contents of the first part of the duodenum.

The curve representing the excess neutralizing ability closely parallels that of the duodenal acidity (fig. 5). It is this excess neutralizing ability of the contents of the first part of the duodenum that accounts for the phenomenon of a rise in acid content without a proportional decrease in pH. The error due to dilution which was operative in causing false negative free acid readings in some duodenal samples is likewise active in this determination. It was demonstrated, however, that when the initial pH of the unaltered sample was above 3.5 the effect of dilution on the hydrogen

ion concentration was slight (1). Since the average pH in the duodenal bulb during the post-meal phase was about 4.5 (fig. 3), the altering effect of dilution would only influence a comparatively small percentage of the observations. The corrected values, undoubtedly, would tend to depress the curve to some extent but the excessive neutralizing ability of this part of the duodenum would still be easily apparent.

**DISCUSSION.** In a previous study one of us (15) has collected data similar to those reported here but following a full meal of raw lean beef instead of the Ewald meal. In the previous study no fasting samples were taken and samples taken within 10 minutes after feeding were not included but this slight difference does not alter the general character of the results. A comparison can, therefore, be made to show the relation of the type and quantity of food to the acidity of the gastric and intestinal contents.

As might be expected, the acidity as measured in pH units of the gastric contents was much higher after the poorly buffered Ewald meal, most of the samples being more acid than pH 2.0 with the peak of the distribution curve falling between pH 1.0 and pH 1.5. In contrast, after the meat meal most of the samples were less acid than pH 2.0 with the peak of the distribution curve near pH 2.6. Samples collected from the duodenum after the Ewald meal were scattered over a wider pH range and showed less tendency to concentrate around a characteristic value than those collected after the meat meal. Distribution curves constructed from the two sets of data both have peaks near pH 4.0 but the curve obtained after the Ewald meal has a second, higher peak, near pH 6.0, a value attained in only two samples following the meat meal. In general the acidity of the intestinal contents was definitely less after the Ewald meal than after meat.

From this comparison the conclusion can be drawn that the acidity of the duodenal contents in the normal dog is largely determined by the type of food undergoing digestion and bears little relation to the acidity (in pH units) of the gastric contents.

Both this and the previous study mentioned above show that in the pyloric antrum as well as in the first part of the duodenum acid is more or less quantitatively buffered as rapidly as it is produced. As a consequence, the pH tends to remain stable in the stomach and to vary in the duodenum independently of the titratable acidity. The first part of the duodenum, in particular, is equipped with a remarkable neutralizing, buffering and diluting capacity considerably in excess of its physiological needs with respect to the free acid of the gastric chyme. Similar conclusions were reached by Imes (5) and by Stevens (14) using a different approach in a study of the reaction of the duodenal contents in parts other than the first part alone.

The consistent difference in pH and titratable acidity of the samples collected simultaneously from just above and just below the pylorus is

so striking as to indicate the existence of a special mechanism whereby the gastric contents are partially neutralized the moment they enter the duodenum. As suggested earlier by one of us (15), such a mechanism may be found in the "receptive relaxation" of the duodenum. Inhibition affecting chiefly the first portion of the duodenum may cause an accumulation of duodenal contents in the vicinity of the pylorus at the moment of exit of the gastric contents, and thus facilitate the quick dilution and partial neutralization of the chyme.

These observations also have a bearing on the question of the occurrence in the intestine of acidities adequate to serve as a stimulus for the regulation of gastro-intestinal functions such as gastric tone and peristalsis, pyloric tonus, and pancreatic secretion. Available evidence indicates that free acid (pH 3.0 or below) in the intestine is needed to affect gastric functions significantly (18, 19) and a pH of 4.0 or below to cause more than minimal stimulation of the pancreas (20). Although acidities below pH 3.0 occurred in our experiments, they were infrequent and were not, in our opinion, present with sufficient regularity or of sufficient duration to account alone for the regulation of gastric emptying. On the other hand acidities of pH 4.0 or below were found in 32 per cent of the duodenal post-meal samples and, therefore, may have been a significant factor in stimulating the flow of pancreatic juice.

#### SUMMARY AND CONCLUSIONS

1. There is a consistent difference in acidity as indicated by pH measurements of samples collected simultaneously from just above and just below the pylorus in normal dogs fed an Ewald meal.

2. Free acid is usually but not constantly absent in the contents of the first part of the duodenum.

3. Acid that is produced in the stomach is more or less quantitatively buffered in the pyloric antrum and as it enters the duodenum it is quickly diluted and partially neutralized.

4. The contents of the first part of the duodenum in the normal dog display a neutralizing, buffering and diluting capacity apparently in excess of the physiological needs.

5. Although titratable total acidities follow roughly a parallel course in the antrum and duodenal bulb, pH values in both regions vary independently of these and of each other. Likewise, variations in titratable free acid in the stomach are not regularly accompanied by corresponding changes in duodenal pH. Consequently, no measure of gastric acidity can be used as a reliable index of the effective acidity (hydrogen ion concentration) of the duodenal contents in the normal dog.

6. Following a meal consisting chiefly of carbohydrate, the acidity of the intestinal contents is rarely sufficient to affect gastric motility significantly but may be a factor in stimulating pancreatic secretion.

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# THE METABOLISM OF FRUCTOSE BY THE EVISCERATED RAT<sup>1</sup>

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Corkill and Nelson (1939) found by analysis of samples of blood and muscle that all the fructose injected at a constant rate for four hours into the surviving, decapitated and eviscerated cat could be accounted for at the end of this time if it was assumed that the sugar was uniformly distributed throughout the blood and muscles and that these composed respectively one-sixteenth and one-half of the total weight of the preparation. This seemed convincing evidence that fructose was not acted on by this preparation, from the circulatory system of which all abdominal organs including the kidneys had been removed. This result, however, was opposed to the findings of McGuigan (1908), Steinberg (1927), Griesbach (1929) and Bornstein and Volker (1929), all of whom showed that the isolated, perfused, hind limb of the dog would remove fructose from the perfusion fluid and therefore at least implied that this sugar was metabolized in the absence of the abdominal viscera. Griffiths and Waters (1936) had also found that fructose prolonged the life of the eviscerated dog, again indicating utilization, but these experiments may have been influenced by the presence of the kidneys which were not removed.

*The destruction of fructose by the nephrectomized, eviscerated rat.* In view of the contradiction implied in the reports mentioned and since the chief weakness of the work of Corkill and Nelson lay in their indirect estimation of the fructose content of their preparation, it seemed desirable to reinvestigate the problem from their viewpoint, but using the rat, which is small enough to be analyzed in toto.

Adult, male rats were eviscerated (the alimentary tract from esophagus to lower colon, pancreas, spleen and liver were removed) after a preliminary, partial ligation of the vena cava (Markowitz and Soskin, 1927) and then nephrectomized in such a manner as to leave the suprarenals. The animals were given 200 mgm. of fructose and the same amount of glucose via the saphenous vein immediately after the completion of surgery and at intervals as indicated for each animal in figure 1. The glucose was given to make certain that the animals would live long enough to allow their tissues ample time to act on the fructose if they were so

<sup>1</sup> Excerpt from thesis presented to Graduate School of the University of Minnesota in partial fulfillment of the requirements of the Ph.D. degree in physiology. Supported in part by a Sigma Xi Grant-in-Aid. Assistance in the preparation of these materials was furnished by the personnel of the Works Progress Administration, Official Project no. 665-71-3-69, Subproject no. 355.

able. After two or three injections the animals were allowed to go on to death without further sugar. Blood samples were taken from the tip of the tail throughout the period of survival, and each sample analyzed for both fructose (Reinecke) and total sugar. The total sugar analyses were made by a modified form of Jeghers and Myers' method (1930) which was found to give the same values for fructose and glucose. The difference between the fructose and total sugar values of a sample was taken as its glucose content.

Immediately after death each animal was dropped into enough hot water containing 1 equivalent of sulfuric acid to make a total volume, including that of the animal's carcass, of approximately 1 liter, and digested by boiling under reflux for 4 hours. These digests were analyzed for fructose as follows: a 10 ml. aliquot was measured into a 100 ml. graduated cylinder containing 20 ml. of 10 per cent

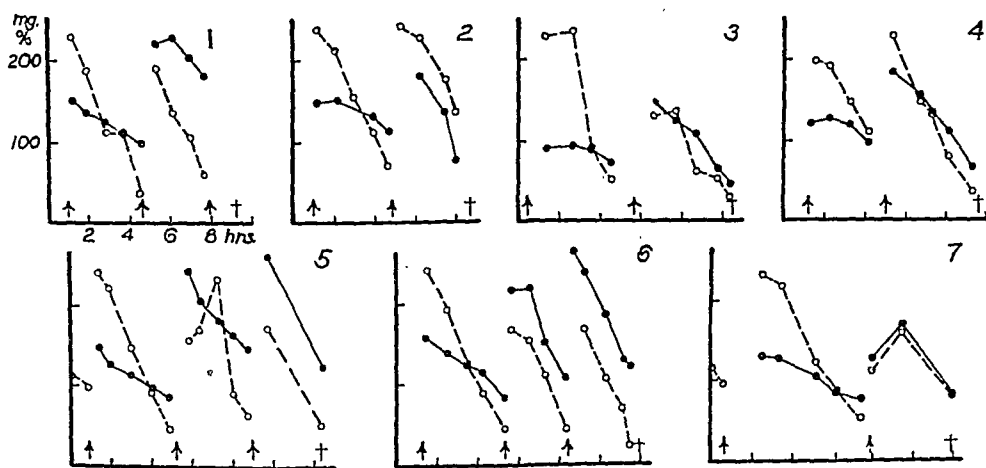


Fig. 1. The blood fructose and glucose levels of eviscerated, nephrectomized rats after the intravenous administration of these sugars. Solid lines indicate fructose levels, broken lines glucose levels. Each arrow indicates the injection of 200 mgm. of fructose and a like amount of glucose. The crosses indicate the death of the respective animals. The numbers in the upper right hand corners identify the animal concerned for comparison with figure 2, group A.

zinc sulfate and a drop of phenolphthalein. Water was added to make 50 ml. and then a saturated barium hydroxide solution to a faint but permanent pink endpoint. Finally more water was added to make 100 ml. and a portion centrifuged. The supernatant fluid was decanted and acidified with a trace of concentrated sulfuric acid. If barium sulfate was formed, it was removed by recentrifugation. Fructose was determined in the clear fluid by the method mentioned. Analyses of controls which had not received fructose showed only a small amount of material giving a reaction for fructose. Analyses of similar controls which were killed within a few minutes after receiving fructose showed that the method detected slightly more than 50 per cent of the fructose present. That the missing fructose was destroyed by the acid treatment is shown by the disappearance of slightly less than 50 per cent of known amounts of fructose boiled under reflux with normal sulfuric acid for the same length of time (see

fig. 2, groups B, C and D). It was felt that such drastic treatment was desirable in order to avoid the objection that fructose stored in some form capable of being rehydrolyzed to fructose might have been missed.

Figure 1 shows that concentration in the blood of both the fructose and glucose in these animals decreased with time, the rate of decrease of fructose sometimes approaching that of glucose. That the decrease in the blood fructose levels reflected a real loss of fructose from the entire system of tissues was shown

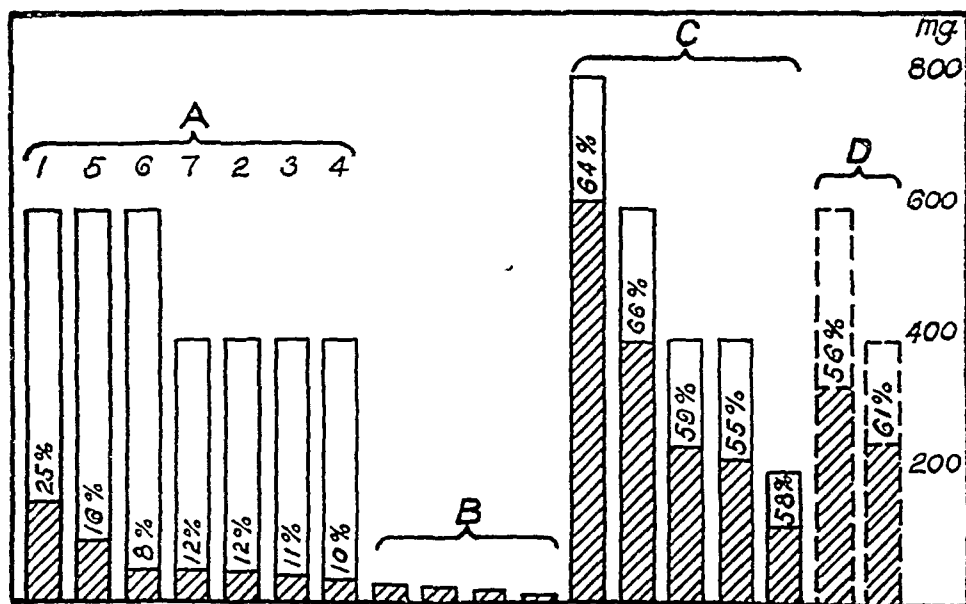


Fig. 2. Analyses of rat carcasses for fructose. The total height of each bar indicates the quantity of fructose added to the system, the shaded portion the amount accounted for by analysis. The percentage accounted for is given at the top of each shaded bar. Group A gives the analyses of the animals whose blood sugar curves are given in figure 1. The numbers above the bars identify them for comparison. Group B gives analyses of controls given no fructose, group C of controls killed a few minutes after the injection of known quantities of fructose. Group D shows the results of the analysis of solutions containing known quantities of fructose in normal sulfuric acid which had been boiled under reflux for the usual digestion period. These analyses were carried through the complete procedure, but did not differ significantly from analyses on aliquots in which the treatment with zinc sulfate and barium hydroxide was omitted.

by the results of the carcass analyses (fig. 2, group A) which accounted for much less than the 50 per cent of fructose injected as found in the controls. In fact the amount of fructose detected in some cases approached that found in the controls which had received no sugar. The highest proportion found, 25 per cent, was in an animal which died about an hour after an injection of the sugar.

*The influence of fructose on the length of survival of the eviscerated rat.* The disappearance of fructose from the tissues of the eviscerated, nephrectomized rat does not in itself mean that it is metabolized in such a way as to be effective in supporting the life of the various tissues of this preparation. Of the reports men-



tioned above indicating that tissue systems can act on fructose in the absence of the liver and intestine only that of Griffiths and Waters, showing that it prolongs the life of the eviscerated, non-nephrectomized dog, implies directly that it is used in the support of tissue metabolism. Therefore experiments were carried out in the eviscerated rat to determine whether or not fructose would also prolong the life of this preparation. These were carried out on groups of 4 adult, male, eviscerated rats, 2 nephrectomized and 2 with kidneys intact. The animals were fasted for approximately 24 hours before being eviscerated. They were given 2 ml. of saline intravenously immediately after the completion of the surgical procedures. One hour later, one nephrectomized and one non-nephrectomized animal were given 0.8 ml. of 50 per cent fructose intravenously followed by 0.25 ml. of saline. The other two were each given 1.05 ml. of saline. All of these fluids were given through a slender rubber tube, anchored at the back of the

TABLE 1

*The survival time in minutes after the completion of surgery of eviscerated rats treated and not treated with fructose*

GROUP	NEPHRECTOMIZED		NON-NEPHRECTOMIZED	
	Fructose not given	Fructose given	Fructose not given	Fructose given
I	95	107	232	444
II	120	82	303	883
III	93	159	397	574
IV			415	675
V*			485	795
			795	995

\* Blood samples were obtained at intervals from the tips of the tails of all animals in groups I-IV, care being taken to obtain the samples in the same way and at the same relative time for all animals within a group. No blood samples were taken from the animals in group V which may account for their markedly longer survival.

neck by a suture and leading under the skin to a cannula tied in a jugular vein. The amount of fluid injected was sufficient to cause the excretion of urine in the non-nephrectomized animals. The animals were then allowed to go on to death without the administration of further saline or sugar. The survival times of the animals are shown in table 1. It is felt that only animals within a group should be compared, for the experiments on different groups were carried out on different days, and it was found impossible to control all of the conditions of the experiments precisely from day to day.

The table shows that the non-nephrectomized animals receiving fructose consistently lived longer under the circumstances than did their controls. It also shows that other things being equal the non-nephrectomized lived longer than the nephrectomized, eviscerated rat. The survival times of the nephrectomized animals were too short and too irregular under the conditions of these experiments to allow the drawing of any conclusions as to the effect of fructose in these ani-

mals. Therefore they were not included when the experiments were performed on the later groups.

DISCUSSION. The finding that fructose disappears from the eviscerated, nephrectomized rats seems to be contradictory to the findings of Corkill and Nelson; but it is to be pointed out that they were working with a different species, performed a somewhat different type of experiment and estimated the amount of fructose remaining in their preparation by an indirect means. The reports of the workers using the perfused hind limb of the dog are supported.

The prolongation of life of the eviscerated, non-nephrectomized rat by the injection of fructose supports the similar finding of Griffiths and Waters for the dog and indicates the probability that fructose is metabolized in such a way as to support the life of tissues, at least in the non-nephrectomized, eviscerated rat.

The rapid demise of the nephrectomized as compared with the non-nephrectomized, eviscerated rat may be explained by the finding of Bergman and Drury (1938) that it was necessary to inject glucose more rapidly into a nephrectomized or anuric, non-nephrectomized, eviscerated rabbit to maintain its blood sugar at a given level than into a similar animal with functioning kidneys. The excretion of urine by all the non-nephrectomized animals makes it unlikely that a difference in kidney function could explain the longer survival of the non-nephrectomized animals receiving fructose as compared with their controls.

The results of these experiments neither support nor contradict the finding of Bollman and Mann (1931) that fructose would not prevent or relieve the symptoms of hypoglycemia in the eviscerated, non-nephrectomized dog or that of Maddock, Hawkins and Holmes (1939) that it would not return to normal the electroencephalogram of the hypoglycemic, eviscerated, non-nephrectomized rabbit. In the studies of these investigators apparently no effort was made to detect any difference in length of survival of eviscerated animals injected with fructose as compared with controls receiving no sugar, or to account for all the fructose at the end of the experiment. On the other hand, in the experiments here reported no attempt was made to prevent the ultimate occurrence of hypoglycemic symptoms by continued injection of fructose into the non-nephrectomized preparation or to relieve them after they had begun. These symptoms, however, did not occur as soon in the animals receiving fructose as in those not receiving this sugar.

#### SUMMARY

Evidence has been presented which indicates that the eviscerated, nephrectomized rat can destroy fructose.

Fructose was found to prolong the life of the eviscerated, non-nephrectomized rat.

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# THE EFFECT OF THE URETHANE OF BETA METHYLCHOLINE CHLORIDE UPON THE PARASYMPATHECTOMIZED CAT'S EYE

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Shen and Cannon (1) demonstrated that excision of the ciliary ganglion produces a pupil which is sensitized to instilled acetylcholine. This finding was confirmed by Keil and Root (2) who also found that a similar phenomenon follows intracranial section of the oculomotor nerve. According to the latter investigators the contraction of the iris sphincter in response to a given dose of acetylcholine reaches a maximum in about five days, and similar curves are obtained until at least the eighteenth day. After this time the responses decrease until they reach a steady minimum within approximately thirty-five days. The recovery of acetylcholine sensitization which occurs when the drug is injected following physostigmine instillation as well as certain observations in the literature (2) suggest that the loss of sensitization in parasympathetically denervated pupils is associated with an increased choline esterase activity.

It should be possible to test this hypothesis by injecting a drug which possesses the muscarine-like activity of acetylcholine, but which is not destroyed by choline esterase. Such a substance was sought among the choline derivatives, for these are known to vary in activity and stability (3, 4, 5). At the suggestion of Dr. Hans Molitor, the urethane of beta methylcholine chloride<sup>2</sup> (hereafter designated Ubm) has been used. This substance has ten times the muscarinic activity of acetylcholine, and it is said to be stable in the presence of choline esterase (6).

**METHODS.** All operations were carried out with strict aseptic precautions on cats anesthetized with nembutal (36 mgm. per kgm. body weight, intraperitoneally). Denervation of the sphincter was accomplished by excision of the ciliary ganglion (1).

At various intervals after operation the responses of the circular muscle of the iris to the intravenous injection of Ubm were observed. Changes in pupillary size were determined by measuring the horizontal diameter with a millimeter scale. From time to time the pupil on the side of the ciliary ganglionectomy was tested for regeneration by instilling into the conjunctival sac two drops of a one per cent solution of physostigmine. When physostigmine instillation produced constriction of the pupil seven days after removal of the ciliary ganglion, the

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<sup>2</sup> The authors wish to thank Dr. Hans Molitor and the Merck Institute for Therapeutic Research for supplying the urethane of beta methylcholine chloride used in these experiments.

excision was assumed to be incomplete. A positive physostigmine effect six weeks or more after ciliary ganglionectomy suggested regeneration of the parasympathetic innervation. Experiments in which there was any suspicion of incomplete removal of the ganglion, or regeneration of the parasympathetic nerve fibers were discarded.

**RESULTS.** The normally innervated cat's pupil constricted when 1 or more mgm. per kgm. body weight of Ubm were injected intravenously (3 cats). Two to six hours after the drug was injected the miosis disappeared. The injection of these large amounts of Ubm produced a pupillary constriction of the parasympathetically denervated pupil at a rate and to a degree that was nicely reproducible for a period of at least seven to eight weeks. In two cats the time required for the maximum contraction of the sphincter increased slightly in the later curves. This change was never great.

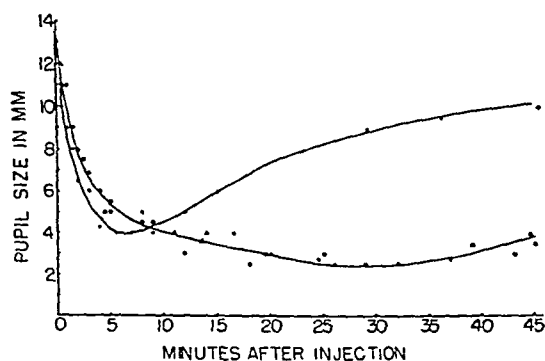


Fig. 1

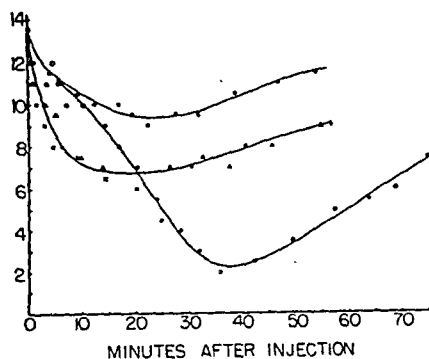


Fig. 2

Fig. 1. The response of the left pupil to the intravenous injection of the urethane of beta methylcholine chloride (115 gamma per kgm. body weight) 8 (+), 15 ( $\Delta$ ), 21 ( $\bullet$ ), and 28 ( $\circ$ ) days after excision of the left ciliary ganglion.

Fig. 2. The responses of the left pupil to the intravenous injection of acetylcholine (5 mgm. per kgm. of body weight) and of the urethane of beta methylcholine chloride (150 gamma per kgm. of body weight). The acetylcholine was injected 8 ( $\circ$ ) and 34 ( $\bullet$ ) days after excision of the ciliary ganglion; the urethane derivative, 12 ( $\times$ ) and 44 ( $\Delta$ ) days after the operation.

The injection of smaller quantities of Ubm (100 to 200 gamma per kgm. of body weight) produced a contraction of the denervated sphincter (9 cats), whereas little change occurred on the normal side (paradoxical pupillary constriction). Figure 1 shows the pupillary constriction which is induced by the intravenous injection of 115 gamma per kgm. of body weight of Ubm 8, 15, 21 and 28 days after excision of the ciliary ganglion. It is important to note that the curves obtained 15, 21 and 28 days after the operation show no diminution in the magnitude of the response. This was true also in cats in which after ciliary ganglionectomy the test injections were carried out for a period of six weeks. In the four animals in which the first test injections were made between 3 and 8 days after excision of the ciliary ganglion, the rate of pupillary constriction and the rate of recovery were more rapid than was the case in the later curves (see fig. 1). There is no obvious explanation for this phenomenon.

The minimum dose of Ubm necessary for the production of contraction of the denervated sphincter varied from animal to animal. Thus, the injection of 120 gamma per kgm. of body weight produced no pupillary constriction in one cat, but 240 gamma induced a good response. In a second animal 94 gamma of the drug were without effect, whereas 185 gamma resulted in a large constriction. In four other cats test doses of 100, 115, 136 and 137 gamma per kgm. of body weight were adequate amounts of Ubm for the production of reproducible pupillary constriction. These results indicate that in cats the threshold probably varies between 90 and 125 gamma of Ubm.

Since the above experiments show that the constriction of the parasympathetically denervated pupil which follows the intravenous injection of a given dose of Ubm can be reproduced for several weeks, and since the responses of a similar preparation to intravenous test injections of acetylcholine decrease about three weeks after ciliary ganglionectomy (2), it seemed of considerable interest to study the effect of the injection of these drugs upon the same denervated sphincter. Figure 2 shows that the pupillary constriction produced by the intravenous injection of 5 mgm. of acetylcholine per kgm. of body weight was much less 34 days after ciliary ganglionectomy than the response to the same dose which was obtained 8 days after the operation (2). On the other hand, the pupillary constriction which follows the intravenous injection of 150 gamma per kgm. of body weight of Ubm was as great 44 days after parasympathetic denervation of the eye as that obtained 12 days after ciliary ganglionectomy. This experiment demonstrates that the contraction of the denervated sphincter of the cat's eye which follows the intravenous injection of Ubm can be induced with undiminished intensity at a time when the response of the same pupil to the intravenous injection of acetylcholine has decreased.

Certain systemic reactions were observed immediately after the intravenous injection of Ubm. Clonic and tonic convulsions appeared only when the doses injected were greater than 1 mgm. per kgm. of body weight. In no instance was artificial respiration necessary. Salivation and lachrymation were more intense and more prolonged than when acetylcholine was injected. Dyspnea was present, but sweating of the foot pads was not observed. Urination and defecation occurred when larger doses of Ubm were injected.

**DISCUSSION.** The constriction of the normally innervated pupil in response to the intravenous injection of large doses of Ubm indicates that this drug possesses a miotic action which is more intense than that of acetylcholine (2). Indeed, the miotic activity of Ubm appears to be comparable with that shown by natural muscarine (7). Since the normally innervated pupil constricts when large doses of Ubm are injected, denervation sensitization can be demonstrated only when small quantities of the drug are administered. Under these circumstances the denervated sphincter contracts, whereas little or no change occurs on the normal side (paradoxical pupillary constriction).

The miotic activity as well as the intense salivation and lachrymation which follow the injection of Ubm indicate that this drug has a more intense muscarine-

like action than does acetylcholine. In addition, Ubm is said to be stable in the presence of choline esterase (6). The difference in stability of the two drugs could explain the finding that Ubm produces a constriction of the parasympathetically denervated pupil which can be obtained with undiminished intensity at a time when the response of the same pupil to the intravenous injection of acetylcholine has decreased. This observation supports the conception that the decrease in acetylcholine sensitivity which occurs three weeks or more after ciliary ganglionectomy is related to an increased activity of the esterase system (2).

#### SUMMARY

1. The normally innervated cat's pupil constricts when one or more mgm. per kgm. body weight of the urethane of beta methylcholine chloride are injected intravenously (3 cats).

2. The intravenous injection of 100 to 200 gamma per kgm. of body weight of the urethane of beta methylcholine produces a constriction of the parasympathetically denervated pupil (9 cats), whereas little change occurs on the normal side (paradoxical pupillary constriction).

3. The intravenous injection of an adequate dose of the urethane of beta methylcholine chloride produces pupillary constriction curves which, between two and six weeks after ciliary ganglionectomy, do not vary significantly in the degree of contraction of the sphincter, or in the rate at which the contraction occurs.

4. The minimum effective dose of the urethane of beta methylcholine which produces constriction of the denervated sphincter probably varies between 90 to 125 gamma per kgm. of body weight.

5. The contraction of the denervated sphincter of the cat's eye in response to the intravenous injection of an adequate dose of the urethane of beta methylcholine can be produced with undiminished intensity at a time when the response of the same pupil to the intravenous injection of acetylcholine (5 mgm. per kgm. of body weight) has decreased (fig. 2).

6. These experiments support the conception that the decrease in acetylcholine sensitivity which occurs three or more weeks after ciliary ganglionectomy is related to an increased activity of the choline esterase system.

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# THE CHANGES PRODUCED ON THE OXYGEN AND CARBON DIOXIDE CONTENT OF ARTERIAL AND VENOUS BLOOD OF THE BRAIN DURING DIATHERMY THERAPY FOR GENERAL PARESIS

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On the basis of his results with artificially induced fever in dogs Hartman (1, 2) has concluded that a constant and severe anoxia occurs which results in brain lesions similar to those of a prolonged asphyxia or carbon monoxide poisoning. He found that in all but one of his experimental animals the oxygen saturation of the arterial blood fell below normal and that the five animals in which the saturation fell below 65 volumes per cent all died. To overcome this anoxia he suggests that oxygen should be administered during fever therapy.

We have studied the changes produced in the blood constituents from 12 patients during diathermic fever treatments for general paresis. The determinations were made on blood taken from the radial artery and the internal jugular vein before therapy started, at the end of two and a half hours when the temperature reached 106°F., and after holding the temperature at this level for 3 to 4 hours. The following factors were studied: oxygen, carbon dioxide, sugar, lactic acid, total protein, specific gravity, hematocrit values, and pH. The pulse, respiration and blood pressure were measured and the rectal temperature recorded continuously on a Leeds and Northrup recording thermometer. In all but three instances the determinations were repeated. Two of these patients died prior to a second experiment. The results obtained on these two patients will be discussed later.

Readings were obtained in duplicate on oxygen and carbon dioxide of both venous and arterial blood for twenty-one experiments and in twenty of these the oxygen capacity was also determined. The means and standard deviations for the three readings of the variables studied are given in table 1.

It will be noted that from the first to the second reading there was a slight fall in the oxygen content of both the arterial and venous blood samples, but that the third reading showed an increase to an even higher level than at the initial reading. The oxygen saturation showed a similar fall and rise but the final value remained lower than the initial level. Part of the increase in oxygen content for the third reading was due no doubt to the increased hemo-concentration as the hematocrit values showed an increase from 40.01 per cent to 43.04 per cent which indicated a 7.5 per cent loss of fluid from the vascular system. As the



temperature was raised there was also an increase in the variability of the readings for both the absolute level of oxygen and the per cent of saturation in the arterial and venous blood samples. This change in variability was most marked in the values for percentage saturation of the arterial bloods taken at the first and second readings, the values for the standard deviations during this time increasing from 6.96 per cent to 14.59 per cent.

The carbon dioxide content of both the arterial and venous blood samples showed a progressive fall amounting to 4.16 volumes per cent for the former and 4.31 volumes per cent for the latter.

The initial temperatures of the patients were quite uniform as indicated by the low values for the standard deviations. That the temperatures were held within

TABLE 1  
*Means, standard deviations and ranges for all variables for three periods*

		FIRST READING			SECOND READING			THIRD READING		
		Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range
Temperature, °F.....		98.41	0.700*	97.4-100.8	105.95	0.389	105.0-106.5	105.92	0.358	105.2-106.6
Pulse .....		73.9	14.00	56-112	112.1	18.21	76-150	116.3	15.32	88-148
Respiration .....		18.8	3.68	14-28	23.9	5.63	16-38	23.4	4.42	16-34
Blood pressure, mm. Hg.....	Syst.	118.5	17.21	86-156	126.5	13.15	106-144	110.4	13.65	88-130
	Diast.	64.9	18.11	54-100	36.0	29.62	0-78	38.9	32.47	0-96
Carbon dioxide, vol. per cent .....	Art.	51.27	3.29	44.58-57.50	49.86	4.05	42.61-59.63	47.11	3.14	39.64-51.49
	Ven.	57.21	3.51	49.66-62.92	56.28	5.26	44.78-67.78	52.90	4.68	38.29-59.02
Oxygen, vol. per cent.....	Art.	16.28	2.08	12.41-19.59	15.81	3.03	10.25-20.88	17.01	3.03	10.69-21.89
	Ven.	10.25	2.64	5.34-17.24	9.85	3.08	6.02-18.09	11.47	3.51	7.94-22.48
Oxygen capacity, vol. per cent.....		18.16	2.54	14.63-23.35	18.90	2.19	14.42-22.77	19.84	2.69	14.50-24.70
Oxygen, per cent saturation .....	Art.	90.20	6.96	72.7-100.0	83.60	14.59	45.1-100.0	85.90	11.29	53.6-100.0
	Ven.	58.10	13.38	32.0-97.3	51.10	14.60	20.2-88.5	57.70	14.17	37.5-92.7
Hematocrit per cent	Ven.	40.01	3.53	32.2-44.6	41.42	4.19	33.8-47.2	43.04	5.42	32.0-49.9
A-V difference, carbon dioxide, vol. per cent.....		-5.95	1.94	-9.95- -0.78	-6.42	3.54	-12.24- +0.68	-5.79	3.23	-10.23-0
A-V difference, oxygen, vol. per cent....		6.03	2.59	10.39-0	5.97	3.22	11.57-0.32	5.55	3.35	11.59-0

\* The standard errors may be calculated from these values by dividing by  $\sqrt{n-1}$  or in these experiments by 4.47.

very narrow limits during the period of elevation is shown by the almost identical means and standard deviations for the last two periods. In only two of the 38 readings were values below 105.5°F. obtained, one a reading of 105.0° and the other 105.2°. The upper limit of temperature exceeds 106.3° in only three instances. During the period of elevated temperature the variability was about one-half that found for the initial reading.

As the temperature was elevated the mean pulse rate increased from 73.9 to 112.1, and then to 116.3. The rate showed a significant positive correlation with temperature with a "r" of 0.709 and a probability of such a relationship occurring by chance of less than 1 in 100. This was a change of 5.1 beats per degree F. as compared with 7 beats found by Simpson (3). Even holding the temperature at this level for 3 to 4 hours caused no significant difference in the result as the value for the rate of change for the final reading was 5.6 beats per degree F.

Coincident with the change in pulse rate the mean systolic blood pressure also showed an increase of 8 mm. Hg for the period during which the temperature was increasing but this was followed by a marked fall during the period of elevation so that the final reading was 8 mm. Hg lower than the initial value. While the increase in temperature caused an increase in systolic blood pressure it had the opposite effect on the diastolic pressure which showed a drop from 64.9 mm. to 36.0 mm. from the first to the second readings and a slight rise to 38.9 for the final reading. The high standard deviations in the periods of elevated temperature were due to the fact that six patients gave at least one reading in which the diastolic pressure was listed as zero. These low pressures were undoubtedly caused by the extreme vasodilatation of the peripheral vessels which permitted a rapid fall in the blood pressure during diastole since the resistance to the flow of blood became negligible. As a result of these diverse effects on systolic and diastolic pressures the pulse pressures showed a very large increase from 53.6 mm. Hg before treatment started, to a maximum of 90.5 mm. Hg during the second reading. For the final reading some slight deleterious effect of the prolonged elevation of temperature is shown in the drop in the systolic pressure to a value somewhat lower than that of the control value. This produced a change in the pulse pressure to 71.5 mm. Hg which was still considerably higher than the initial value. The mean respiration rate also increased during the period of fever from 18.8 at the first reading to 23.9 for the second reading and 23.4 for the final reading. The coefficient of correlation between temperature and respiration was 0.52 and the Fisher's *p* for this occurring by chance was less than 0.01. The blood velocity was not measured directly but it is evident from the increases in pulse rate and pulse pressure that the velocity was markedly accelerated during the period of fever.

The differences between the arterial and venous levels for carbon dioxide and oxygen are also given in table 1.

DISCUSSION. Our results on the effect of elevated temperatures on oxygen utilization do not agree with those reported by Himwich and his co-workers (4). They found that in 11 of 15 experiments the oxygen A-V difference rose from 2.0 volumes per cent to 8.1 volumes per cent. We found that the mean oxygen A-V difference changed from 6.03 volumes per cent for the control period to 5.97 volumes per cent for the second period and 5.55 volumes per cent for the final period. Of the 21 cases the second reading was higher than the first in 10 instances. There was no significant difference between any of the means, but there was a tendency to a slight but progressive decrease in the utilization of oxygen as the temperature was kept elevated. There was no significance in the differences between the arterial and venous values of carbon dioxide for the three readings but it is to be noted that the changes in carbon dioxide did not coincide with those of oxygen as the greatest difference was obtained between the first and the second periods. It is evident therefore that this change in carbon dioxide did not arise from an increase in metabolism. That the increased rate of respiration has resulted in a washing out of carbon dioxide is shown by the progressive fall in both arterial and venous carbon dioxide content amounting to 4.16 volumes per cent for the former and 4.31 volumes per cent for the latter. The R.Q. as cal-

culated from the blood gas differences changed from 0.988 for the initial reading to 1.075 for the second reading, and 1.043 for the final reading. This would seem to indicate that the formation of fatty substances was accelerated in the brain during the period of elevated temperature.

Two of the patients studied in this series died. One of these deaths may possibly have been due to the diathermy treatment. He was given a single treatment during which his temperature rose from 98.2 to 106.2 within an hour and remained elevated for 4 hours. His pulse increased from 56 to 76 and then to 98 beats per minute and his blood pressure first rose from 138/68 to 145/50 and then fell to 100/0. The respiration rate increased from 18 to 24 and then to 28 per minute. The arterial blood oxygen levels were 18.02, 18.41 and 18.95 volumes per cent for the three samples and the corresponding percentages of saturation were 93.3, 90.1 and 92.7. The venous blood-oxygen levels were 10.78, 18.09 and 18.95. The pH values were 7.32 for venous and 7.37 for arterial at the beginning, 7.44 for both at the second period, and 7.42 for both at the third period. It is evident therefore that despite the fact that his blood was being oxygenated sufficiently in its passage through the lungs, it was not delivering any oxygen to the brain cells. This is also shown by the fact that the pH of the venous and arterial bloods were identical for the last two periods. The change in carbon dioxide content of the blood also substantiates this. The initial reading gave 57.04 volumes per cent for venous blood, and 47.51 volumes per cent for arterial blood. The next two readings show no significant difference between the arterial and of the venous bloods, the values for the second reading being venous 46.88 volumes per cent and arterial 46.38 volumes per cent, and those for the final reading being 45.92 and 45.82 volumes per cent. The day following this treatment the patient had an N.P.N. of 60.0 mgm. per cent, 6 days later the level was 98.4 mgm. per cent and 8 days later just before his death the value was 142.0 mgm. per cent. The autopsy confirmed the finding of death from uremia. Unfortunately permission to examine the brain could not be obtained.

In the second case the death occurred from bronchopneumonia 52 days after the diathermy treatment which was the second one that the patient had taken. This patient showed a progressive decrease in the percentage saturation of the arterial blood from 94.2 per cent to 89.0 per cent and finally to 66.7 per cent. He also showed a decrease in the utilization of oxygen by the brain from 10.39 volumes per cent for the first period to 6.52 volumes per cent for the second period and no utilization during the third period. In this last period the pH of both venous and arterial blood had the same value, 7.54. It is questionable that this death can in any way be attributed to the diathermy treatment, but it is interesting to note that the two patients who died were the only ones who gave identical values for the pH of venous and arterial blood.

We have not been able to confirm the findings of Hartman (2) that oxygen saturation of arterial blood below 60 per cent results in death. In four instances we found arterial oxygen saturation values below 60 per cent. None of these patients showed any ill effect from the treatment and the patient who died as

the result of treatment had an oxygen saturation of over 90 per cent in his arterial blood.

In only 6 of 42 readings taken during fever was the arterial saturation below 70 per cent and the mean value for this period was approximately 85 per cent. Furthermore the decrease in the amount of oxygen supplied to the brain was not significant. This finding casts doubt upon the advisability of following Hartman's (2) suggestion that oxygen should be administered during fever therapy. The possibility that a partial anoxia occurs in the brain and other tissues during fever therapy and that in some cases this may be severe enough to result in death is not ruled out by these experiments but this cannot be controlled by inhalations of oxygen. The patient whose death may be attributed to the treatment certainly showed no utilization of oxygen by the brain during the treatment. However, more damage seemed to be caused to the kidneys than to the brain. A possible explanation for the effect on the kidneys may lie in the placement of the electrodes around the trunk so that a concentration of the heating effect occurred in the region of the kidney.

#### SUMMARY

In twelve patients receiving diathermy treatments for general paresis the oxygen and carbon dioxide levels of arterial blood and of blood from the internal jugular veins were determined. The temperature was raised to 106.0°F. and maintained at this level for 3 to 4 hours. No significant change in the mean A-V values for either carbon dioxide or oxygen was found. The mean oxygen saturation of the arterial blood decreased from 90.2 per cent to a minimum of 83.6 per cent. There was no evidence of increase in brain metabolism shown by the changes in the blood gases. A significant fall in the mean carbon dioxide levels of both arterial and venous blood occurred.

The administration of oxygen to patients during diathermy treatments does not seem to be warranted as the oxygenation of the arterial blood in the lungs is not dangerously lowered.

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# AN ANALYSIS OF THE ACTION OF ACETYLCHOLINE ON THE CARDIAC GANGLION OF LIMULUS POLYPHEMUS<sup>1</sup>

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The following report deals with the response of the heart of *Limulus polyphemus* to certain choline derivatives, notably acetylcholine; it adds to the rapidly accumulating data which must be correlated in the ultimate evaluation of the rôle which this substance plays in the process of stimulation and transmission—especially its rôle in ganglionic activity.

This study has been made by the general methods used by Carlson (3) in his study of the action of drugs on this organ. This heart is neurogenic and the ganglion, owing to its superficial location in the dorsal median line of the posterior muscular segments, may be treated with solutions either *in situ* or after it has been dissected free from underlying tissue. The anterior (first and second) segments possess no rhythmogenic nerve cells, normally exhibit no inherent rhythm and contract solely in response to impulses from the ganglion. These anterior segments, therefore, like a nerve-muscle preparation, may be directly studied for effects upon the neuro-muscular transmission and graphic records of their contraction serve to quantitate the muscular responses to the efferent impulses from the ganglion.

Studies were made with acetylcholine, either as bromide or chloride, with acetyl-beta-methylcholine chloride ("methyl") and with carbaminoylcholine chloride ("lentin," "doryl").<sup>2</sup> Their effects were compared with the action of choline bromide. The drugs were dissolved in blood plasma or in sea water—in a few instances in Van't Hoff's solution. It was found that solution of the drugs caused only slight changes in pH which were wholly without physiological significance. All solutions were freshly prepared for each experiment and careful checks were made to give assurance that the hydrolytic decomposition during the time course of any experiment was quantitatively negligible. The responses of eighty-nine hearts were observed.

**NEURO-MYAL TRANSMISSION.** The anterior segments were treated with the desired solution by immersion, by lavage, or as in some of our experiments by perfusion through the lumen. Special precautions were taken to avoid all

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<sup>2</sup> The author is indebted to Merck and Company for an adequate supply of crystalline "lentin".

possible contamination of the posterior ganglionated portion of the heart and thus to limit the action of the solutions strictly to the neuro-myal structures. This can be done either with the heart *in situ* in the animal or with that organ removed from the body. All methods gave identical experimental results which may be briefly summed in the statement that neither acetylcholine nor the other choline derivatives mentioned above when applied to the muscle alone had the slightest stimulating effect either upon the rate or the height of contraction of the normally beating heart (fig. 1), even when the concentrations in the sea water or Van't Hoff's solution were excessively high (1 in 100). The slight weakening of the muscular contraction, sometimes noted, is quite in contrast with the stimulating effect of the solution diluted forty times (1 in 4000) when applied to the isolated ganglion of the same heart, for, as is usually the case with this

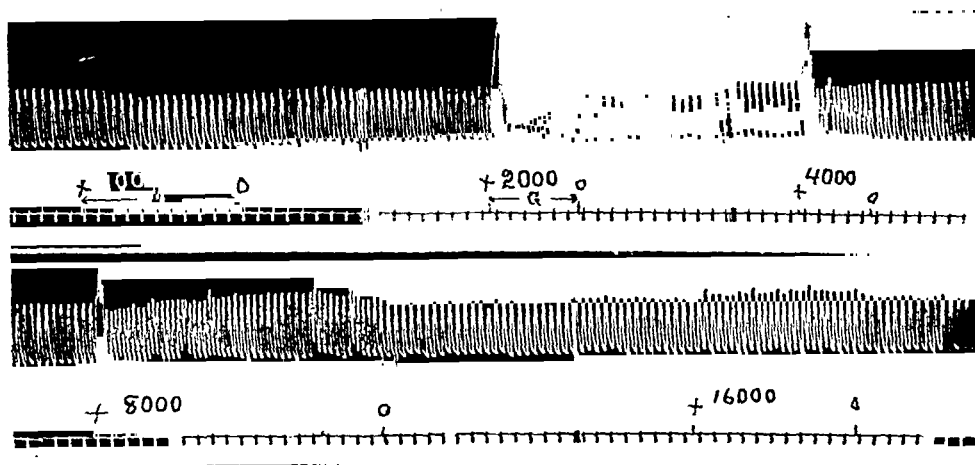


Fig. 1. Effects of acetylcholine on the heart of *Limulus*. The numerals indicate the dilution of 1 part of acetylcholine in sea water. + indicates the application and O the removal by washing with sea water. One part in 100 was applied to the muscle—M—of the anterior segments without physiological results. The more dilute solutions, 1 part in 2,000, 4,000, 8,000 and 16,000, were applied successively to the ganglion—G—and produced stimulation. No potentiation. Time trace, lower line—6 sec.

concentration, there was a sharp tetanic contraction of the muscle and accelerated rhythm; the decreased height of contractions often noted in the latter instance are secondary to the faster rate and are not due to ganglionic depression.

The above effects of acetylcholine were obtained in our earlier experiments without attempts at potentiation with eserine or prostigmine which is so essential to the stimulating effects of acetylcholine in the presence of cholinesterase. Contrary to the statements of Bacq (2), this specific enzyme has been demonstrated in Arthropods in the blood, skeletal muscle and heart muscle of *Limulus* by Smith and Glick (12) who have found the cardiac ganglion of *Limulus* to be especially rich in cholinesterase.

The experiments were therefore repeated in an effort to potentiate the acetylcholine effects with eserine and with prostigmine. These drugs were applied to the muscle of the anterior segments in concentrations far greater than was

necessary to inactivate all cholinesterase (1 in 1000 eserine) and subsequently the muscle was treated with acetylcholine bromide solutions in concentrations varying from one in one hundred thousand to one in one thousand without eliciting any alteration in the height of the normal muscular contractions. Similarly when acetylcholine and the potentiating drug were present in the same solution (sea water) there was no potentiation of contractions, nor did they elicit the characteristic contracture exhibited by the dorsal muscles of the leech and the rectus abdominis muscles of the frog.

The above series of experiments was repeated upon the severed anterior segments of the heart. Contractions were elicited by electrical stimulation of the dorsal median nerve fibers, a preparation in every way comparable to an ordinary nerve-muscle preparation. The muscle was immersed in solutions of the drugs, either separately or simultaneously, and induction shocks of various strengths and sequential groupings were applied to the dorsal median nerve of the preparation. Without further detail it may be stated that in no instance was there the slightest evidence of potentiation, or in fact of any action of acetylcholine on the contractions. Mecholyl and lentin were likewise without augmenting effect upon the muscular contractions which were induced by electrical stimulation of the nerve. Corroborative evidence is to be found in Carlson's (3) statement that physostigmine in high concentration (1 in 500) was without physiological effect on its contractions when applied to the heart muscles and further confirmation is found in the fact that mecholyl and lentin (doryl) likewise fail to enhance contractions of these muscles although they are practically unaffected by the hydrolytic action of cholinesterase. From these results it is obvious that the presence of cholinesterase is not *prima facie* evidence that the acetylcholine mechanism is present. This enzyme plays no rôle in the neural stimulation of the cardiac muscle of *Limulus*. Likewise, on the basis of the above results, acetylcholine can be eliminated from consideration as a chemical mediator of the nerve impulses to the heart muscle of *Limulus*.

As a corollary to these conclusions we may add that the above experiments indicate conclusively that there are no functionally rhythmogenic nerve cells in the first two segments, a fact long ago demonstrated by Carlson (1900) and abundantly supported by many observations since that time. The following sections will show that, were such nerve cells present, they would have been stimulated and aroused to rhythmic activity by the action of the choline esters used in the experiments recounted above.

**EFFECTS UPON THE GANGLION.** The ganglion was subjected to the action of the solutions of choline esters by lavage, by immersion of the posterior segments with its ganglion *in situ*, or by similar treatment of the ganglion after dissecting it free of the underlying muscle but retaining connection with the untreated anterior muscular segments, the contractions of which served as an index of ganglionic activity. Further details of method will be omitted since all methods gave identical effects both qualitatively and quantitatively. In effective concentrations, all the choline derivatives caused initial stimulation of the ganglion, an effect which was sustained in solutions of moderate strength but with stronger

solutions there was frequently a subsequent decrease of rate and weakening of contractions which may be attributed to inhibitory action of the drug from which, however, there was ultimate recovery in sea water if the effect was not too profound. Inhibition as an initial effect of these drugs was never observed. In these features the action of the cholinesters was similar to that observed by Carlson (3) with other drugs which are known to inhibit the vertebrate heart such, e.g., as muscarine, pilocarpine, physostigmine and nicotine. In the experiments to be described we are not concerned with peripheral innervation but solely with effects upon the ganglion. By analogy with effects on the vertebrate sympathetic ganglia the effect of dilute solutions would be the "nicotine effect."

When acetylcholine was used without any previous treatment which might induce sensitization or potentiation, our experiments showed that 69 out of 73 tested for the liminal stimulation value of acetylcholine showed effects only when the concentrations were greater than one part in 16,000. In general the concentrations required were one in 10,000 of sea water, although eight of these hearts showed no response until the strength was one in 5,000 or greater. Of four very sensitive hearts, one showed a response of a limiting dilution of approximately 120,000, one at 50,000, one at 36,000 and one at 25,000. These concentrations are excessively high in comparison with those which are necessary to produce other known physiological effects of acetylcholine; for example, inhibition of the vertebrate heart (1 in many millions) and in almost unbelievable dilution ( $1:10^9$  even  $1:10^{12}$ ) it will inhibit the heart of the marine clam, *Venus mercenaria* (11). The conclusion that the ganglion of *Limulus* lacks sensitivity to drugs, based on the above results, is negatived by Carlson's (3) evidence that while its stimulation requires relatively high concentrations of some drugs the ganglion can be stimulated by high dilutions of nicotine (1 in 5 million), aconitine, veratrine and adrenalin.

Figure 1 shows a series of tracings illustrating the effects of acetylcholine described above. In it one may note that even 1 to 100 acetylcholine failed to stimulate the muscle or the myoneural junctions of the second segment of the heart, while decreasing concentrations  $\frac{1}{20000}$ ,  $\frac{1}{40000}$  and  $\frac{1}{80000}$ , when applied to the ganglion initiated a transient tetanus followed by an accelerated rate with its consequent reduction in height of contraction. A dilution of  $\frac{1}{16000}$  caused mild stimulation noted in an increase of both rate and height of contraction. The stimulating effects of a  $\frac{1}{32000}$  dilution were just detectable. This liminal concentration must be looked upon as physiologically excessive but it may safely be said the ganglion of this particular heart would not have responded to it had it not experienced previous treatment with the stronger solutions of the drug. For most hearts the threshold for stimulating effects of lentin was reached at dilution approximating 1 part in 300,000.

LENTIN (carbaminoylecholine chloride, "doryl"). Our own experiments show that the ganglion is especially sensitive to lentin (doryl), a compound closely related to acetylcholine, but one not hydrolyzed by cholinesterase. In general it may be said that lentin has more than ten times the stimulating activity of unpotentiated acetylcholine; frequently the individual ratio was as high as 25 to



1; to give a specific example, the most sensitive ganglion of our series responded to one part of acetylcholine in 120 thousand of sea water but a similar stimulation was induced by lentin, 1 part in 3 million. Lentin needs no potentiation, in fact cannot be potentiated by eserine, but lentin by itself has stimulating powers which closely approximate those of acetylcholine which has been potentiated with eserine (see below). There are certain differences, however. Strong solutions do not produce so stormy and powerful a tetanus, contractions are more regular, and augmentation of height usually accompanies the increase in rate, there being less tendency to the tonic contraction which raises the relaxation base.

Mecholyl chloride is a choline ester very slightly hydrolyzed by cholinesterase. It cannot be potentiated by eserine. Unlike lentin it has very slight stimulating value for the ganglion, possibly half that of unpotentiated acetylcholine and lacks entirely the "nicotine effect" of the latter drug. The base choline (bromide) also will stimulate the ganglion but is still less effective than mecholyl. Owing to the great variability in the stimulation threshold of different heart preparations it is impossible to fix definite values for the relative stimulating effects of these different preparations.

*Potentiation to acetylcholine action.* Reference has already been made to the fact that the cardiac ganglion of *Limulus* has a very high content of colinesterase (Smith and Glick, 12). It is well established that this enzyme, by hydrolytic decomposition of acetylcholine, can reduce materially or even abolish the physiological effects of this drug. Most effective in "paralyzing" this enzymic action are eserine (physostigmine) and prostigmine which thus "potentiate" the action of acetylcholine. This potentiating effect of eserine is especially well exemplified by its action on the *Limulus* heart ganglion. Although eserine itself does not stimulate the ganglionic discharge of the ganglion until it is present in concentrations greater than one part in 8,000 of sea water, solutions for more dilute than this, e.g., one in 50,000 or even one part in 100,000 of sea water (one part in 20,000 was commonly used in our experiments) leave the ganglion highly sensitive to subsequent dilutions of acetylcholine which without this potentiation have no detectable effect. The potentiation by eserine sets in almost instantaneously, and the effect of a single treatment for a few seconds may still be demonstrated several hours later in spite of persistent effort to wash the structure free from every trace of the potentiating drug. Prostigmine acts in a similar manner although there are quantitative differences which will not be dwelt with at this time. The effects of potentiation with eserine are illustrated in figure 2. In this experiment acetylcholine (1 in 10000) without potentiation produced no acceleration and 1 in 7500 merely effected a slight elevation of the basal relaxation tonus. Eserine alone in a concentration of 1 in 10,000 caused only mild stimulation (a 10 per cent increase in rate), but this single treatment profoundly affected the response to subsequent treatment with acetylcholine as is illustrated by the marked stimulation induced by this drug at a dilution of 1 in 50,000, a stimulation which is still exhibited with progressive dilution to 1 in 300,000.

Evidence of the marked potentiating action of eserine and the fact that this effect is wholly independent of its own stimulating value is presented in the trac-

ings in figure 3. In this illustrative experiment, on a highly sensitive preparation, both the eserine and acetylcholine were present in the stimulating solutions in equal concentrations, that of each being far below its individual stimulating value for the ganglion. While many other experiments demonstrated stimulation by such solutions when the concentrations were 1 in 100,000, in the example given above dilutions of 1 in 200,000 and 1 in 400,000 also produced marked stimulating effects.

*Eserine and pilocarpine.* The examples of stimulation shown in figure 3 serve another purpose; they indicate that eserine acts in a very specific way which is not the result of its own stimulating power. Its effectiveness is demonstrable

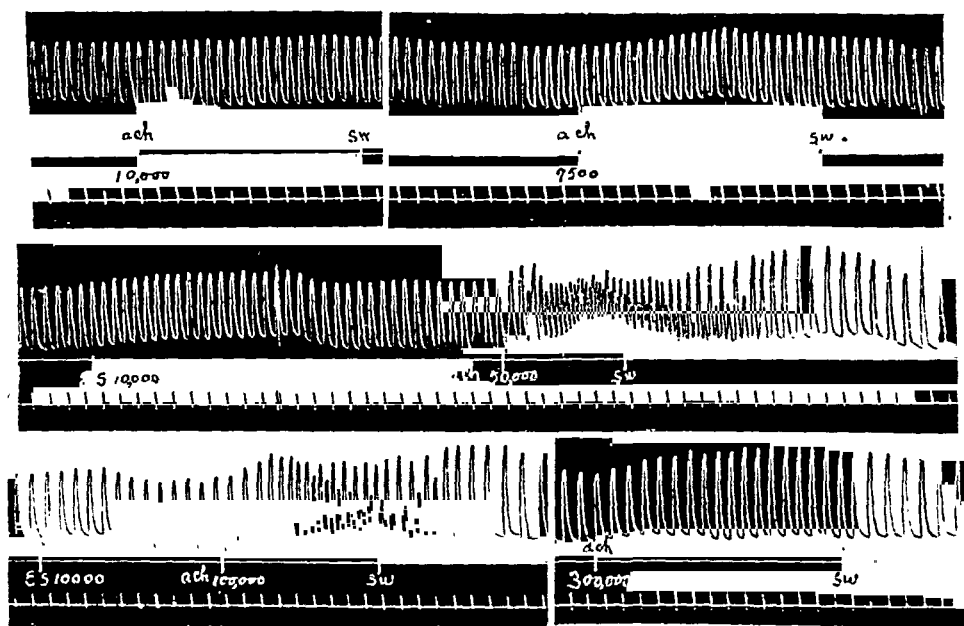


Fig. 2. Effects of potentiation of acetylcholine solutions with eserine upon their stimulation of the cardiac ganglion. Acetylcholine solutions 1 in 10,000 and 1 in 7,500 (upper tracing) produced only mild stimulation. Eserine (1 in 10,000), middle tracing, was only mildly stimulating but after its application, the stimulation by acetylcholine was demonstrable in high dilutions: 1 part in 50,000 (middle tracing) and 1 part in 100,000 and 300,000 respectively (lower tracing).

only in conjunction with acetylcholine. It does not have the slightest potentiating effects even in conjunction with choline or other choline esters such as mechohyl or lentin; this is an indication not only of its ineffectiveness as a direct stimulating agent at these dilutions but that its effect, furthermore, is not due to a rise in the threshold of stimulation, i.e., to an increase in the sensitiveness of the preparation to other stimulating agents. This pronouncement is best illustrated by the experiment reproduced in figure 3. The individual liminal stimulating concentrations for pilocarpine, eserine and acetylcholine differ very slightly for a given ganglion and in this preparation was a dilution of about 1 in 10,000 as shown in A and B of the figure. In C it may be seen that pilocarpine of this

liminal concentration does not potentiate a 1 in 10,000 solution of acetylcholine; neither does the combination of eserine and pilocarpine at D give any indication of enhancement of the stimulating power of either drug, but when eserine and acetylcholine (1 in 10,000) act together (E) a powerful irregular tetanus results, followed by an augmented rhythm, then a slow rate and final return to high regular contractions (not shown). This specific effect of the combination of these two drugs was evident with dilutions much greater than 1 in 400,000 (G); the same effect as that illustrated in figure 2.

**ATROPINE.** The action of this alkaloid was studied on account of its well known effect in "blocking" the action of acetylcholine in certain of its peripheral

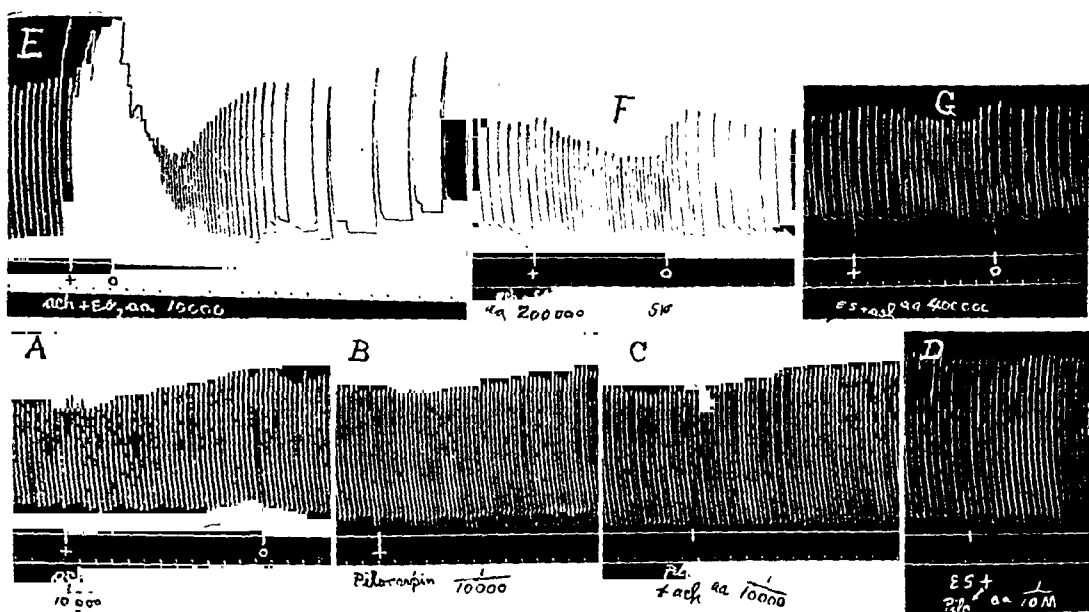


Fig. 3. In dilution of 1 part in 10,000 acetylcholine applied to the heart ganglion was only mildly stimulating (A) as was the same dilution of pilocarpine (B). The combination of these two agents at this dilution added little to the individual stimulation of either drug (C) and at (D) it is demonstrated that eserine, 1 part in 10,000, does not enhance the action of pilocarpine. The upper tracings show the marked stimulating action of equal parts of eserine and acetylcholine in dilutions of 1 in 10,000 (E), one in 200,000 (F) and 1 in 400,000 (G). + and O mark application and removal of the drugs, respectively.

effects such as vagal inhibition of cardiac muscle. Upon the cardiac ganglion of *Limulus* it has no such effect, its only action in non-toxic concentrations being stimulation, as Carlson (3) pointed out. The liminal concentration for this effect lies between 1 in 8,000 and 1 in 5,000. This stimulating effect increased with increasing concentration and could be summed with that of other stimulating drugs such as eserine and pilocarpine, but notably with unpotentiated acetylcholine itself, the effect of which upon the ganglion was never inhibited by atropine, thus comporting itself as it does on the sympathetic ganglia of vertebrates.

These facts emphasize the difference between the mechanism of inhibition of

the vertebrate heart and that of *Limulus*. In the latter, inhibition is restricted to the ganglion, which however is stimulated by acetylcholine, the peripheral motor neuromuscular function being unaffected by acetylcholine, while in the vertebrate heart it is the musculature which is inhibited by the action of the drug on parasympathetic nerve endings. In the ganglion there is no decrease in the stimulating power of potentiated acetylcholine. Figure 4 illustrates these points. A shows the stimulating effect of 1 in 2,000 atropine and the superimposed effect of 1 in 2,000 unpotentiated acetylcholine. In B, eserine and acetylcholine had been diluted to 1 in 100,000 and the atropine concentration increased in the common solution to 1 in 500, but the high stimulating potency of the dilute potentiated acetylcholine was not interfered with.

**INHIBITION.** In this connection another point bearing on inhibition should be referred to, namely, that the stimulation of the ganglion by whatever agency used, particularly by potentiated acetylcholine (or by lentin), did not reduce the ease with which ganglionic inhibition could be induced by direct rapid faradiza-

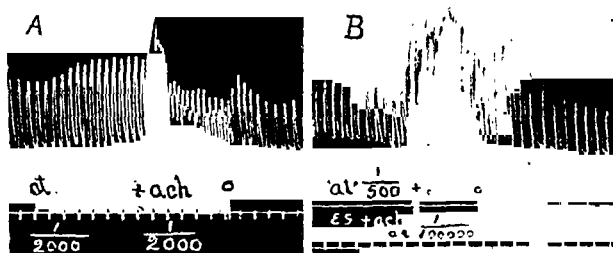


Fig. 4. Atropine is a stimulating agent when applied to the ganglion; a 1 in 2000 solution applied at *at* (A) increased the rate and amplitude of contractions, and stimulation by 1 in 2000 acetylcholine summed with it (+ to  $\circ$ ). Acetylcholine with potentiating eserine, each 1 in 100,000, produce profound stimulation of the ganglion in spite of atropine (1 in 500) B.

tion as described by Garrey and Knowlton (7). In fact the heightened activity of the ganglion actually made it easier to induce inhibition by direct electrical stimulation. This might have been anticipated on the basis of the Garrey and Knowlton observations. It is as if the stimulation by the drugs induced a condition tending to autogenous inhibition and it is possible to so interpret either the weak contractions or the slow rhythm which so commonly accompanies or follows intensive stimulation by acetylcholine (fig. 3). Finally in this connection it should be stated that atropine does not interfere with inhibition of the ganglion however induced, whether by stimulation of ganglionic afferents, as has been done by Carlson, or by rapid repeated induction shocks or condenser discharges (7).

**RESUSCITATION OF GANGLIA.** Hearts which have been left standing in sea water, or *in situ* in exsanguinated animals, often show marked irregularity of rate, with depressed contractions which have lost all semblance of uniformity. It was found in these cases that treatment with solutions of acetylcholine usually restored them to a marked degree. Two examples of this effect are shown in

figure 5, the contractions in A are those elicited by the depressed, exhausted or fatigued ganglion, they showed the immediate pick up which resulted when 1 in 10,000 acetylcholine was applied. Within ten minutes they were restored to regular uniform contractions and (B) the ganglion showed acceleration when treated with 1 in 200,000 acetylcholine after eserine potentiation, indicating a high degree of sensitivity and normal function. A less regular but nonetheless striking improvement in contraction of another exhausted preparation is illustrated in C (fig. 5) due to acetylcholine and also as a result of choline. The improvement remained effective only while these drugs were acting and dis-

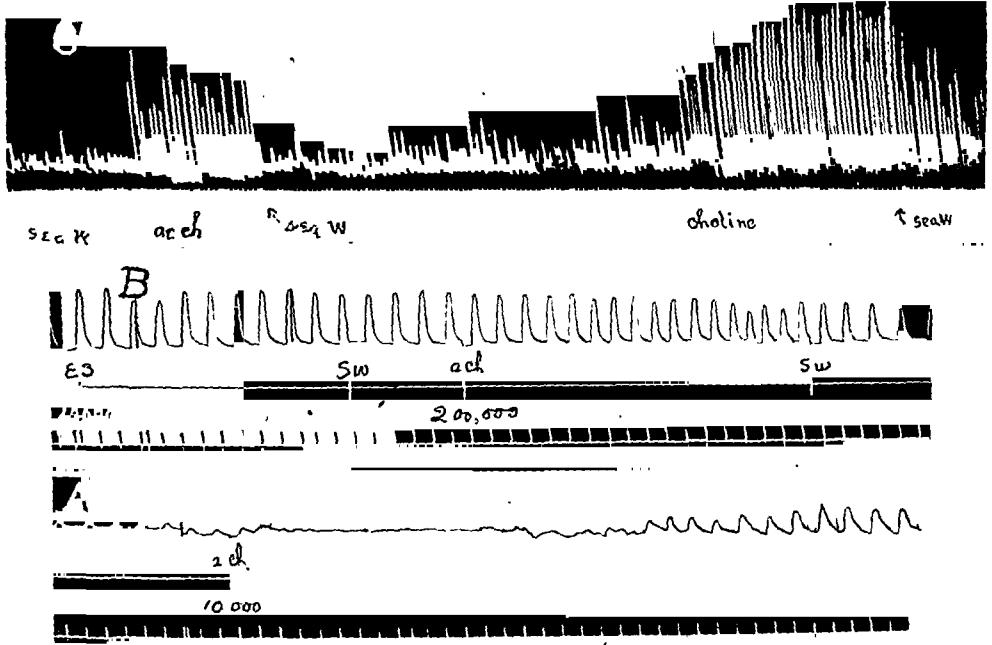


Fig. 5. Showing the muscular contractions initiated by an "exhausted" ganglion, A, and the prompt improvement due to 1 in 10,000 acetylcholine, illustrated by B; treatment with 1 in 10,000 eserine at *es* potentiated the ganglion to subsequent treatment with 1 in 200,000 acetylcholine solution which caused definite acceleration. The upper tracing (C) shows the improvement of contraction in another badly functioning ganglion by treating it with acetylcholine solution at *ach* and later also with a choline solution. Time tracing in 6 second intervals.

appeared when they were washed away. Had the treatment been continued it seems probable that restoration with complete regularity would have resulted. If vertebrate nerve centers are responsive to similar treatment these reactions assume a considerable significance. An earlier communication by Garrey (5) has pointed out that the only mechanism by which an increase in the height of contractions of the *Limulus* heart muscle can be accounted for is recruitment, i.e., the increase in the number of motor neurons delivering effective impulses to the muscle. The resuscitating effects then can only mean that within the ganglion more motor cells respond to the rhythmic impulses from the pace maker or, in

other words, either the receptive motor neurons or their synapses became more excitable as a result of the action of choline and its esters. This response will be referred to in a succeeding section. The results bear a striking resemblance to the neuromuscular augmentation, described by Garrey and Knowlton (6), which results from subliminal or very mild stimulation of the intracardiac nerve fibers and suggest that the augmentation described above is due to a stimulating action of acetylcholine on the motor cells of the ganglion and possibly is related indirectly to the "spontaneous background" discharge described by Armstrong, Maxfield, Prosser and Schoepfle (1).

SEGREGATION OF THE EFFECTS OF ACETYLCHOLINE STIMULATION. The outstanding expressions of stimulation are the increase in rate and the increase in the height of the contractions. While application of acetylcholine to the entire ganglion usually results in an increase in both rate and height it is usually possible

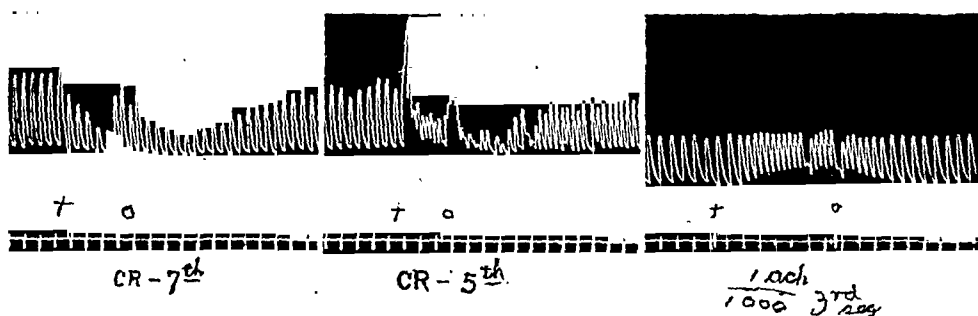


Fig. 6. Determination of pacemaker by localized stimulation with acetylcholine, in the left hand section, a tiny crystal of acetylcholine bromide was placed on the ganglion in the middle of the 7th segment; the depression of contraction suggest inhibition (cf. text). The stimulating effects, however are illustrated in the middle section showing the effects of a crystal of the drug on the ganglion at the 5th segment. A solution of acetylcholine, 1 in 1,000, localized at a capillary point on the ganglion at the 3rd segment likewise stimulated with a shift of the pacemaker function to the minute point stimulated, without augmentation of contractions.

by selection of the concentration of the drug to increase the height of the contraction without affecting the rate. Heinbecker (8) has postulated that, of the two types of ganglion cells originally described by Wm. Patten (10), the more numerous smaller cells are motor cells to the muscle while the larger are capable of initiating the rhythm. Heinbecker presented a diagram of assumed synaptic connections with the smaller cells. Garrey pointed out (4) that it is possible to dislocate the pace-maker function to any minute locus on the ganglion between the third and seventh or eighth segments by punctate warming of the ganglion. It now develops that the same result may be obtained by similarly restricted application of acetylcholine (best in conjunction with eserine). This is easily accomplished by a capillary applicator containing the solution or by soaking a thread in the solution and laying it transversely across the ganglion or by applying a tiny crystal to the ganglion, cf. figure 6. By these means the rate can be accelerated without increasing the height of contractions of the anterior seg-

ments of muscle; in fact, the increased rate may decrease height of contractions as it does in the vertebrate heart. If while the higher rate is thus maintained adequately, stimulating solutions of acetylcholine or lentin be applied along the length of the ganglion there will result a prompt augmentation of the height of contractions. The increased rate can be interpreted only as a direct stimulation of pace-making nerve cells. The augmentation of the muscular response invites further discussion. It has been pointed out previously by Garrey (5) that the prime factor influencing the height of the muscular contraction is the number of motor nerve fibers discharging impulses to the myocardium, and the augmentation here noted is so interpreted, i.e., the increase in height of contraction is due to recruitment. The normal contractions of the heart are never maximal and it is obvious that not all of the motor cells discharge with each beat; there is a large reserve of non-participating, idle cells and augmentation is the result of transformation of these into participating active cells. The evidence is clear that acetylcholine increases the excitability of all the nerve cells, but the additional factor of synaptic facilitation is strongly indicated by the experiments described above and thus we have a dual mechanism by which the motor cells become responsive to the impulses from the pace maker.

Eserine is known to inactivate cholinesterase and it can readily be seen that this action alone would increase the stimulating action of acetylcholine on the ganglion since the ganglion has a high content of esterase which can hydrolyze the esters more rapidly. It is a striking supplement to this action, however, that the potentiating action of eserine, once induced, may be evident several hours later, in fact has been demonstrated in our experiments as much as fifteen hours after a single treatment of the ganglion with eserine. This fact gives rise to certain questions concerning the relation of esterase and ganglionic action. If the ganglionic function is dependent on the action of normally developed acetylcholine, why is it that esterase potentiation by eserine in itself does not lead to accumulation of autogenous acetylcholine and thus cause a progressively increasing activity of the ganglion?<sup>3</sup> This effect has never been noticed in our extensive experience with its use. Again and conversely if esterase is essential to the restoration of normality after each discharge, as has been maintained by Nachmanson, how account for the fact that eserization of the ganglion by concentrations sufficient to inactivate all esterase does not augment either the height of contraction or the normal rhythmic discharge through the hours during which it can serve as an adequate potentiation agent for acetylcholine? Since the effects of eserine do ultimately wane and disappear, are we to assume a breakdown of an eserine-esterase combination with restoration of the original cholinesterase or are we to assume the development of a new enzyme quantum, and if so, is its origin to be ascribed to neural action? It is to be borne in mind that the ganglion may be dissected free from all other tissue and still maintain its functions by intrinsic action. These questions have yet to be answered before one

<sup>3</sup> F. R. Miller has reported the precipitation by eserine of continuous activity of localized cortical areas of the cat brain resulting in muscular tremors, rigidity and powerful clonus; these effects he attributed to synaptic facilitation.

can be sure that acetylcholine is essential to, or even plays any part in, the normal function of the ganglion.

#### SUMMARY

1. Acetylcholine, even with the potentiating influence of eserine, is without effect in facilitating the transmission of motor nerve impulses to the musculature of the Limulus heart. This is also true for the cholinesters, lentin and mecholyl and for choline (bromide) itself.

2. Acetylcholine, in physiologically strong solutions (*circa* 1 in 10,000) will stimulate the ganglion, effecting an increase both in rate and amplitude of muscular contractions.

3. Eserine potentiates the stimulating action of acetylcholine on the ganglion thus increasing its effectiveness from twenty to fifty times, an effect which may persist for hours. It does not have this effect with lentin or mecholyl.

4. Atropine stimulates the ganglion. It does not prevent the stimulation by acetylcholine nor the potentiating effect of eserine in combination with acetylcholine.

5. Acetylcholine has a profound effect in restoring function to the depressed (exhausted, fatigued) cardiac ganglion of the Limulus heart. Choline has a similar effect.

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# THE EFFECT OF HYPERTONIC PLASMA ON THE BODY FLUIDS IN NORMAL EXPERIMENTAL ANIMALS<sup>1</sup>

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The appreciation of the value of hypertonic plasma in the treatment of shock and other disturbances where an increase in plasma proteins or circulating fluid volume is needed, makes necessary a more comprehensive knowledge of the effects of intravenous injections of hypertonic plasma into normal subjects. The present study is, therefore, an investigation of the changes in body water distribution, electrolytes, and other related factors produced in normal dogs receiving varying amounts of hypertonic plasma.

Freeman and Wallace (1) were able to demonstrate an increase in plasma volume in dogs following the intravenous administration of four times concentrated serum. We (2, 3) have shown that the intravenous administration of concentrated adtevac plasma constantly produces a plasma volume increase in cases of clinical shock and in normal humans. However, the report of Harkins, Boale and Brush (4) is not in complete agreement with these expected results. On the contrary in normal dogs under nembutal anesthesia they demonstrated hemoconcentration following the administration of concentrated plasma.

It is apparent that any change in plasma volume brought about by the intravenous administration of hypertonic plasma is only a part of a generalized shift of body water. The nature and mechanism, as well as the effects of this cellular and extracellular water shift have remained unmentioned and uninvestigated so far.

In this investigation certain complicating factors which deserve special mention made themselves apparent. Reactions due to sodium citrate may be encountered in dogs if 30 to 50 cc. of three or four times concentrated plasma, prepared from citrated blood, is given intravenously. This is, of course, a small dose in adult humans and is entirely without ill effect, but may be attended with temporary reactions in infants and children (2). Thus, the intravenous injection of 80 mgm. of sodium citrate per kilogram of body weight will, in dogs, lead to transient tetany (5) due to the binding of ionic calcium. Vagal paralysis and cardiac standstill (6, 7) may also be produced if sufficient concentration is attained. Reactions consisting of chills and fever (8) are not due to sodium citrate per se (9).

Furthermore, when concentrated plasma prepared from blood collected during

<sup>1</sup> This study was aided in part by a grant from Abbott Laboratories, North Chicago, Illinois.

barbiturate anesthesia<sup>2</sup> is given intravenously, the barbiturate effect becomes a factor. The concentration of the drug in such dog plasma is usually sufficiently low as to produce only mild effects.

Particular efforts were made in these experiments to remove these two complicating factors, but they are considered here in order to emphasize the necessity of taking such factors into consideration in interpreting results of various investigators in the study of the treatment of shock in animals or the effects of administration of concentrated plasma products to normal animals.

The plasma used was first desiccated from the frozen state; consequently denatured protein need not be considered as a complicating factor.

**METHODS.** Normal healthy dogs were used in these experiments. Anesthesia was not necessary. Medium sized animals were used through preference in order that body water changes might be more readily recognized. The desiccated plasma used was prepared by the adtevac process (10). The concentrated solution was prepared by dissolving the dry product in a reduced volume of distilled water to give an average of 15 grams of protein per 100 cc. Both human and dog plasma were used. Citrated plasma and defibrinated plasma were given in different experiments. These variations are noted in the accompanying table. When citrate and nembutal were present in the plasma they were frequently largely removed by dialysis against saline conducted at low temperatures. After a preliminary period of control studies, dogs were given from 3 to 12 cc. of concentrated plasma per kilogram of body weight. The time of injection varied from one to five minutes. Studies at 15 minute, 30 minute, one hour and various other intervals after the administration of plasma were carried out. These studies included red cell counts using equipment checked by the National Bureau of Standards; hemoglobin by the method of Sanford-Sheard (11) using a Leitz-Mass photoelectric colorimeter; and hematocrit studies using Wintrobe tubes (12). Plasma volume studies were carried out by the blue dye method of Gregersen, Gibson and Stead (13). The injection of human hypertonic plasma containing small amounts of hemoglobin and varying amounts of bilirubin modified the color of the plasma so that plasma volume studies could not always be accurately carried out. In these instances it was felt that plasma volume changes calculated from changes in the hemoglobin concentration were fairly accurate. The extracellular fluid volume, i.e., plasma volume and interstitial fluid volume combined, was calculated as fluid available for solution of sodium thiocyanate (14). Sodium thiocyanate was assumed to disappear at the rate of 0.08 mgm. per 100 cc. per hour (14). Plasma sodium was determined by the method of Butler and Tuthill (15), and potassium by the method of Truszkowski and Zwemer (16). The method of Greenberg (17) was used for the determination of total plasma proteins. All blood samples were collected from the jugular veins.

**RESULTS.** The accompanying table is a summary of findings in six of the series of eleven dogs. In the remaining five animals identical results were obtained. It will be readily seen that beginning within fifteen minutes after injec-

<sup>2</sup> Nembutal was used in the present study.

tion of hypertonic plasma, hemodilution quickly reached a maximum, as shown by marked and parallel decrease in red blood cell count, hemoglobin, and hematocrit. In accord with this observation, the plasma volume uniformly increased, proportionately in most instances to the calculated hydrophilic property of the injected plasma. The total red cell mass, determined from plasma volume and hematocrit, shows no appreciable change in those animals where dye studies of the plasma volume were satisfactory, so that the plasma volume increase re-

TABLE 1

DOG NO. AND WEIGHT	PLASMA GIVEN	TIME IN- TERVALS IN MINUTES AFTER CONC. PLASMA INJECTION	RBC IN MILLIONS	HEMOGLOBIN IN GRAMS PER 100 CC.	HEMATOCRIT	MEAN CELL VOLUME	PLASMA VOLUME IN CC.	RBC VOLUME IN CC.	WHOLE BLOOD VOLUME IN CC.	EXTRACELLULAR WATER IN CC.	INTERSTITIAL WATER IN CC.	PLASMA SODIUM IN MCM./ 100 CC.	PLASMA POTASSIUM IN MCM./100 CC.	PLASMA PROTEINS GRAMS PER 100 CC.	TOTAL CIRCULATING PRO- TEINS IN GRAMS	GRAMS OF PROTEIN IN- JECTED IN PLASMA
No. 1 8.5 kilo	50 cc.	Control	6.1	13.9			428			2332	1884	343	16	5.1	22	7.5
	Defib.	20 min.	4.5	11.4			555							5.1		
	Dog	60 min.	4.7	11.4			531			2694	2163	338	16	5.4	28	
		390 min.	5.4										20			
No. 2 9.5 kilo	30 cc.	Control	7.9	17.6	54	68	562	609	1171	3005	2443	339	20	5.8	33	4.5
	Defib.	25 min.	6.9	15.6	47	67	600	521	1121					5.9	35	
	Dog	70 min.	6.5	15.4	46	68	620	594	1216	3280	2660	345	19			
		255 min.	7.2	15.5	49	68										
No. 3 9.0 kilo	70 cc	Control	7.0	14.3	44	63	469	399	869	2948	2479	336		7.1	33	10.5
	Defib.	24 min.	5.4	11.9	36	66	644*	399	1043					7.3		
	Human	55 min.	6.0	13.3	41	68	538*	399	937	3033	2495	343				
		300 min.	5.9	12.4	39	67	601*	399	1000	3472	2871			7.1	43	
No. 4 7.5 kilo	95 cc.	Control	7.0	15.4	48	69	345	362	707	2200	1855	337		6.4	22	14.25
	Dialyz.	45 min.	5.4	11.4	35	67	520									
	Dog defib.	95 min.	5.7	12.7	41	70	520	360	880	2332	1812	343		6.3	33	
No. 5 9.2 kilo	90 cc.	Control	5.0	12.0	35	70	780	440	1220	3861	3080	305				13.5
	Citr.	15 min.	3.5	10.2	29	86	952									
	Dog	60 min.	3.4	10.2	29	86	983	402	1385	4023	3040	320				
		280 min.	4.2	10.8	29	70				4575		288				
No. 6** 7.4 kilo	30 cc.	Control	6.1	13.4	42	69	433	350	783	2170	1737	352		7.75	34	4.5
	Citr.	15 min.	5.8	12.0										8.8		
	Dog	75 min.	5.7	13.4	42	73	470							8.4	40	
		160 min.	5.2		39	75	522	326	848	2291	1769	356		8.4		

\* Blood volume changes calculated from changes in hemoglobin concentration; all other blood studies by Evans Blue dye method.

\*\* Dog 6 received calcium chloride sufficient to neutralize effect of sodium citrate which was given in the plasma.

flected itself as a similar whole blood volume change. This increase in plasma volume was maintained up to the six hours observed. From the table, it will be seen that there was a slight but distinct increase in mean red cell volume following the intravenous injection of hypertonic plasma. The concentration of sodium thiocyanate in the extracellular fluid (plasma) showed an invariable decrease which was apparent from one to four hours following the injection of concentrated plasma. In the table this finding is interpreted as increase in extracellular fluid.

Changes in sodium and potassium in the plasma were of minor character and principally did not exceed the range of error of the methods used.

The concentration of protein in the plasma showed little appreciable change, the calculation of total circulating plasma proteins revealed an increase roughly corresponding to the amount of injected protein.

In regard to the general effect upon these animals receiving concentrated plasma in proportionately very large amounts, it may be stated that no deleterious results whatever from the plasma itself were observed. In those animals receiving citrated plasma—calculated dose of sodium citrate being at least 80 mgm. per kilogram—temporary tetany occurred from which recovery was quick and complete and was associated with no effect upon the studies of water redistribution carried out as above. In animals receiving non-dialyzed dog plasma, mild nembutal effects, as staggering gait, retching and drowsiness were encountered; here too, the results pertaining to body-water changes were unmodified. No important change in heart rate or respiration was observed. An invariable result of concentrated plasma in normal dogs seems to be defecation and urination.

**DISCUSSION.** The increase in plasma volume produced in these normal animals by the injection of hypertonic plasma corresponds with most previous experimental studies (1, 2, 3), but seems contrary to the results obtained by Harkins and others (4). This demonstrated effect establishes a firm basis for the use of concentrated plasma in overcoming oligemia of shock, hemorrhage, etc. The indicators of hemodilution (red cell count, hemoglobin concentration and hematocrit) parallel the plasma volume change and demonstrate that storage or release of red blood cells from storage places, such as the spleen, does not influence the results under these conditions. Consequently, a similar study in splenectomized animals seems unnecessary. The degree of plasma volume increase appears to be dependent entirely upon the osmotic effect of hypertonic plasma since the amount of fluid entering the blood vessels is proportional to the total amount of protein injected.

The two major possible sources of this increased plasma fluid are the interstitial and intracellular water compartments, although fluid in the lumen of the gastrointestinal tract must also be considered.

Various factors influence the shift of water across membranes from one compartment to another. Body cells act as osmotic units, susceptible to changes in the osmotic pressure of their environment, the interstitial fluid (18). If the osmotic pressure in the interstitial fluid be increased, water diffuses out of the cell into the extracellular spaces (18, 19). The converse causes water to diffuse into the cell.

When the plasma osmotic pressure is increased a series of events seems to take place. The processes of osmosis and diffusion rapidly transmit the increased osmotic pressure to the interstitial compartment by the passage of fluid from the interstitial space to the plasma. At the same time ions diffusible through the endothelium pass from plasma to the interstitial water. These mechanisms reestablish the osmotic equilibrium between plasma and interstitial water re-

sulting in a final increased osmotic pressure in both compartments of the extracellular fluid. In order to reestablish osmotic equilibrium between the cells and their environment, fluid then passes from the cell into the extracellular space, increasing the interstitial fluid volume.

We have attempted to measure these variations in the volume of extracellular fluid by changes in the amount of fluid (extracellular) available for solution of thiocyanate and also by changes in the concentration of sodium in the extracellular fluid (since sodium is an ion which is non-diffusible through the cell membrane). The invariable dilution of thiocyanate in one to four hours after the injection of hypertonic plasma seems to be strong qualitative evidence for the occurrence of such an increase of extracellular fluid as postulated above on the basis of body fluid dynamics.

In some experiments there was somewhat greater increase of extracellular water than that expected from the protein concentration alone of the solution injected. Since the hydrophilic effect of protein is dependent on changes in hydrogen ion concentration and the concentration of other cations, the question may not be completely answered by osmotic forces.

The failure of sodium to decrease with this increased extracellular water is probably explained by the additional amount of sodium injected, this being proportional to the increased water in the extracellular compartment.

In view of the findings here reported it becomes apparent that the interstitial and intracellular water compartments constitute large available reservoirs of water for supplementing plasma fluids. Furthermore, the mobility of cellular and extracellular water in this sense takes on added significance in considering the disturbances of body water distribution in shock, hemorrhage, burns, infections with parenchymatous degeneration (intracellular edema) and in the treatment of these conditions.

Although in some instances as in experimental hypertonic saline shock and in hemorrhagic shock with anoxemia (19), the changes in mean red cell volume corresponds with tissue cellular fluid, in these experiments it is apparent that no such correlation exists.

#### SUMMARY

1. Intravenous injection of hypertonic plasma in normal dogs leads regularly to a long sustained increase in plasma volume more or less proportional to the amount of protein injected, the red blood cell count, hemoglobin, and hematocrit decrease indicating a proportional hemodilution. No appreciable changes in size of the red blood cells were produced. The total circulating mass of red blood cells does not seem to be altered.

2. The total extracellular water volume is increased after the injection of concentrated plasma as shown by dilution of thiocyanate. The source of this water is assumed to be mainly from cellular water and the redistribution would appear to depend upon principles of osmosis and diffusion between the cells as osmotic units and their surrounding fluid environment.

3. The availability of cellular water in this manner is considered to be of sig-

nificance in the correction of oligemic states. Also the mobility of cellular water suggests further defects occurring in shock, hemorrhage, burns, infections, etc., and indicates a possible therapeutic attack of such a body water defect.

4. In considering the presumed ill-effects of concentrated plasma in small animals, it should be kept in mind that certain extrinsic substances such as sodium citrate, nembutal, denatured proteins, allergens, etc., may be present in sufficient quantity to produce observable effects. This would seem to be of importance in appraising results in the treatment of experimental shock.

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# COMBINATION OF HYPOXIC AND HYPERCAPNIC STIMULATION AT THE CAROTID BODY

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It is common experience to witness under various circumstances the mutual facilitation of the two common respiratory excitants, carbon dioxide excess and oxygen lack. Recently Dill and Zamcheck (1940) demonstrated quantitatively their additive effects in man, and Gellhorn and Lambert (1939) reported evidence from narcotized dogs of their synergistic effects on blood pressure.

The latter authors also raised anew the question concerning the site(s) or mechanism(s) of mutual facilitation of the two excitants. Their experiments allowed the obviously probable interaction of centrally impinging afferent impulses from the carotid and aortic bodies, with direct central changes caused by CO<sub>2</sub>-excess and O<sub>2</sub>-want. Gesell, Lapidès and Levin (1940) demonstrated the interaction of these "reflexogenic" and "centrogenic" components during eupnea, hypercapnia and anoxemia.

In addition Gellhorn and Lambert interpreted results of their experiments on chemoceptively-deafferented dogs to indicate direct synergistic interaction of hypercapnia and hypoxemia at the vasomotor "center", and reinterpreted results of Selladurai and Wright (1932) and Smyth (1937) to allow of the same for the respiratory center. Dumke, Schmidt and Chiodi (1941), however, do not agree. Although a *potentiation* of the CO<sub>2</sub> response by hypoxemia was apparent after denervation in 5 of their 14 animals, and examination of their mean data for the 14 animals shows that the experienced effect of a combination of hypoxemia and hypercapnia approximated the algebraic addition of the individual separate effects,<sup>1</sup> they draw, it would seem, an unwarranted conclusion that "the additive effects of anoxemia and hypercapnia on respiratory minute volume" in intact animals "were therefore due entirely to chemoreceptor reflexes aroused by the former". This statement, of course, would exclude not only direct addition at

<sup>1</sup> The mean minute-volume of respiration was:

During O <sub>2</sub> -administration.....	4720 cc.
During hypoxemia.....	3470 cc. (-1250 cc.)
During hypercapnia (with high O <sub>2</sub> ).....	8040 cc. (+3320 cc.)
During combined hypoxemia and hypercapnia.....	6580 cc.
Calculated combination effect, on the basis of algebraic addition (4720 - 1250 + 3320).....	6790 cc.

The actual minute-volume during combination of conditions thus deviated from that predicted by algebraic addition of the separate effects by 3 per cent; the *change* in minute-volume (from basal), by 10 per cent.

the "center" which their own data suggest, but also a third possible point of addition—at the peripheral chemoreceptors.

There is now no doubt that both hypercapnia and hypoxemia are capable of chemoreflex excitation of respiration through the carotid bodies. Differences of opinion exist concerning thresholds and potency. Though available evidence indicates that this reflexogenic support of breathing is more important in hypoxia than in hypercapnia, there appear to be no actual data on its contribution during a *combination* of these conditions (asphyxia). So long as the chemoreceptors retain some possible importance in the regulation of breathing in the intact organism, either in eupnea or stress, or both, it seems of interest to report the following data, evidencing a mechanism for *local* addition within them of hypoxic and hypercapnic chemoreflex effects.

The apparatus and methods used are described in more detail elsewhere (Winder, 1937a, b). The vascularly isolated carotid segments were perfused with a heparinized mixture of 52 to 77 per cent defibrinated blood in Locke's solution (except expt. 5, in which 35 per cent whole blood was used), through the common carotid arteries as inlets and lingual arteries as outlets, at approximately mean arterial pressure or somewhat above, by means of a continuous artificial circuit which continuously equilibrated the basal perfusing fluid with a gas mixture containing between 5.95 and 6.06 per cent  $\text{CO}_2$  (in the various experiments), and over 93 per cent  $\text{O}_2$ . Accessory reservoirs, each connected with a collapsible bag containing the proper gas mixture, were used for shunting anoxic, hypercapnic or "asphyxial" fluids into the circuit beyond the aerator, without change in perfusion pressure. The anoxic fluid was previously equilibrated with a gas containing less than 0.5 per cent  $\text{O}_2$  and the same  $\text{CO}_2$  content as the basal mixture (the difference ranged from 0.07 per cent less to 0.12 per cent more; average, 0.016 per cent less); the hypercapnic fluid with 35 per cent  $\text{CO}_2$  and  $64 \pm 1$  per cent  $\text{O}_2$ ; the "asphyxial" fluid with 35 per cent  $\text{CO}_2$  and less than 0.5 per cent  $\text{O}_2$ . Temperature in the circuit was controlled electrically.

Mean (femoral) arterial pressure and perfusion pressure were recorded with mercury manometers, and respiration with a recording spirometer attached to a rebreathing circuit. The vagus nerves were intact.

As indicated in the figures, the experimental fluids were administered for short periods of 25 to 46 seconds (average, 32 sec.). There was considerable volume in the circuit between the point of admittance and the carotid, so that during the whole of such short periods the experimental fluid was undoubtedly diluted with basal fluid before reaching the carotids. That is, the effective potency of the experimental fluids was considerably less than would be implied by the gas mixtures used. However, in any one experiment reported, circumstances were unaltered while the three fluids were being used, so that the responses are comparable with one another with one qualification: Because these procedures were originally intended in another connection, the durations of administration were unfortunately not equal in any one series. Consequently, in each experiment *only the first parts of the responses are quantitatively comparable*. Specifically, for



a given experiment the responses are deemed comparable up through a period equal to the sum of the latent period and the shortest administration period among the three trials; i.e., up to the right borders of the vertical rectangles in the figures. (*Vida* "latent period" and "duration of briefest administration".) The "basal respiration" in each experiment is the mean of points before the beginnings of administrations. The "calculated combination" response is the algebraic addition of the separate  $\text{CO}_2$  and low  $\text{O}_2$  responses, with reference to the basal respiration. The sequence of procedure varied, with the "asphyxial" fluid intermediate in order in experiments 2, 3 and 4.

All the pertinent data, from the five experiments applicable to the present problem, are reported in the corresponding figures. *The addition of the reflex respiratory effects of hypoxia and hypercapnia in combination at the carotid bodies is clear in experiments 1 to 4.* The effect of the actual combination (asphyxial fluid) agrees closely with the calculated effect of the combination of  $\text{CO}_2$  and low  $\text{O}_2$ . There were, of course, uncontrollable irregularities such as occasional deep breaths. Either alone or in combination, hypoxia no doubt altered  $\text{CO}_2$  transport, while hypercapnia altered oxygen transport and oxidations; but such factors in the end only contributed to the total of added  $\text{CO}_2$  and low  $\text{O}_2$  effects:

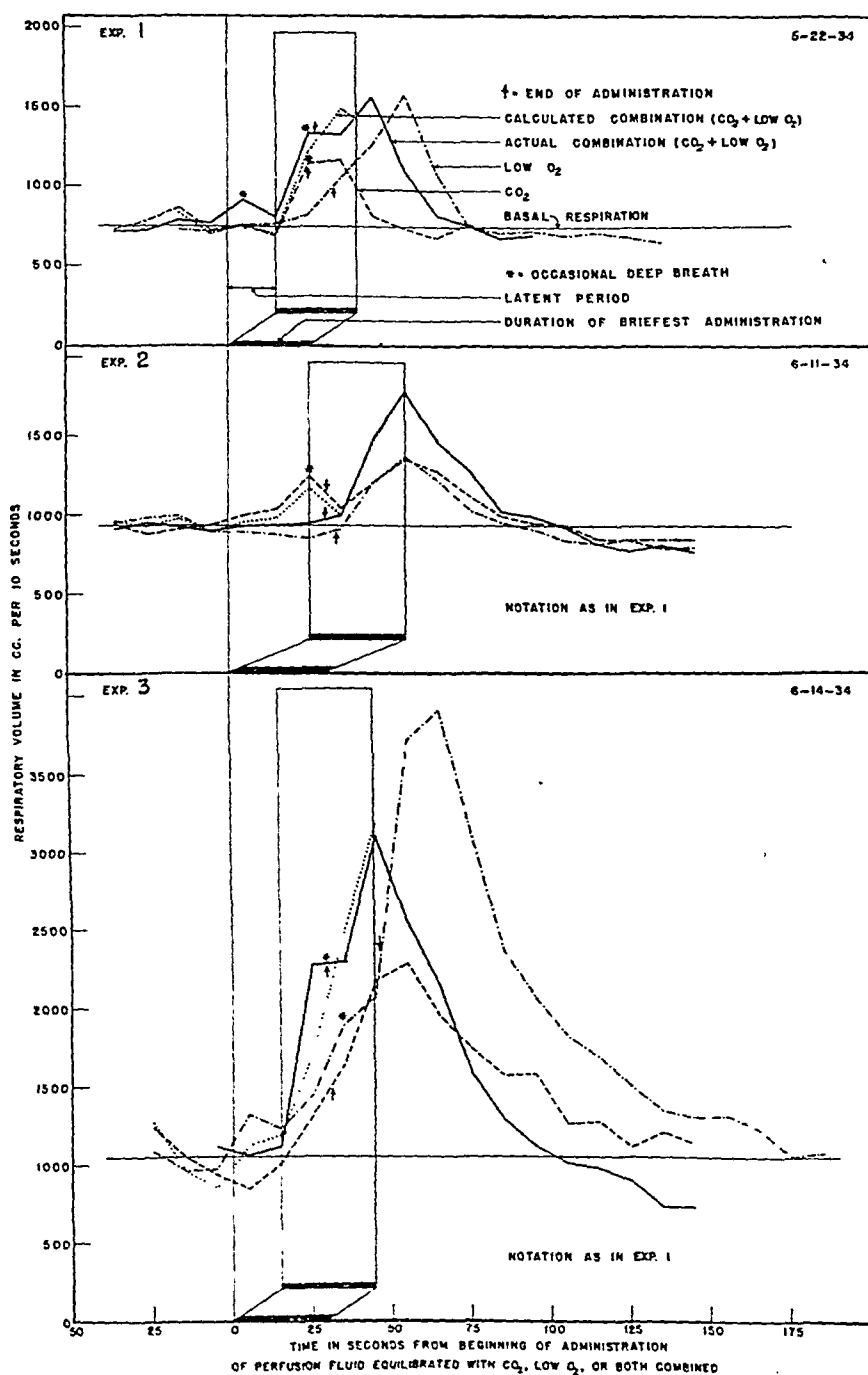
It is recalled that the perfusion fluid in experiment 5 contained least blood, and that in this case alone the blood was not defibrinated. At its face value, the outcome of experiment 5 would appear to be an exception, in that all three procedures yielded approximately the same responses, qualitatively and quantitatively. This fact in itself, however, in the face of differentiation of responses in all the other experiments, probably indicates a *low ceiling* of response of an accidentally poor preparation. This interpretation is further supported by the fact that the maximal responses of all other preparations to the same or more brief administrations considerably exceeded the responses of this one in steepness and extent. Thus, *rather than providing a true exception, the results of experiment 5 apparently express accidental circumstances masking the normal addition of hypoxic and hypercapnic effects.*

It should be pointed out that in these brief administrations the responses were cut short of their maximal. It is evident that they were increasing, usually steeply, at the ends of the comparable periods. That is, the data reported here actually pertain to the summated contributions of hypoxic and hypercapnic stimulation to the *onset* of carotid body excitation. It is probable, of course, that summation in onset is continued in the steady state. Further, these brief procedures offer the probable advantage of providing responses least complicated by secondary changes in the organism, such as hypocapnia.

Concerning the fundamental mechanism through which this summation of stimuli operates, the facts at present either strongly support or satisfy the hypothesis that intracellular acidity, representing interaction between metabolism and immediate environment, is an important circumstance in excitation of carotid body chemoreceptors.

Their internal structure and rich sinusoidal circulation predict an active metabolism (De Castro, 1926, 1927-28). During local chemical suppression of anaerobic glycolysis

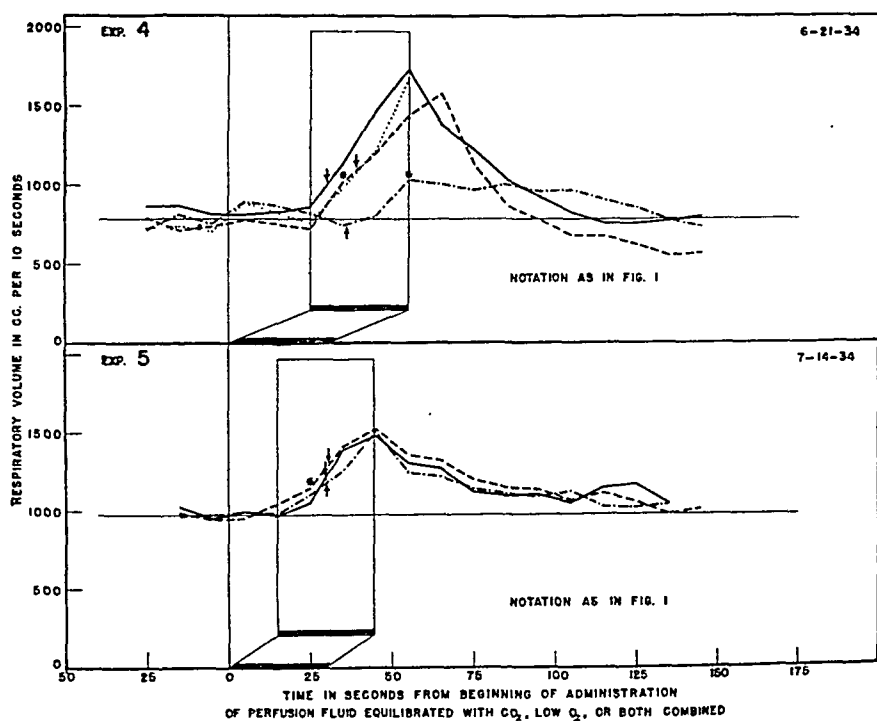
their normally strong hypoxic excitation is replaced by depression while their hypercapnic excitation may persist (Winder, 1937b). Local thermal (Bernthal and Weeks, 1938;



Figs. 1-3

Schmidt, Comroe and Dripps, 1939) or chemical (Shen and Hauss, 1939) increase in metabolism and acidity increases their excitation. During ischemia, cumulative interaction between their metabolism and a static environment results in a strong excitation (Winder,

1938a; Winder, Bernthal and Weeks, 1938; cf. also Bogue and Stella, 1935; Samaan and Stella, 1935; Stella, 1936; Rudberg, 1938, 1940). Their degree of excitation in general parallels their theoretical internal acidity under a variety of chemical conditions (Bernthal, 1938; von Euler, Liljestrand and Zotterman, 1939), and they respond delicately to *exogenous* acidity (Bernthal, 1938; Schmidt, Comroe and Dripps, 1939). Cell cH is very probably not the *only* fundamental factor involved in ultimate stimulation of the receptors. For example, four years ago, in view of the potency of acetylcholine in exciting the carotid body (Winder, 1938b; unpublished experiments indicate that eserization increases its potency to approximately that of nicotine), we proposed that this or a similarly acting material, liberated and protected by metabolic acidity, may possibly be involved in normal transmission of excitation from the epithelioid cells to their contiguous nerve endings (Winder, 1938c; Gesell, 1939, footnote 1).



Figs. 4 and 5

Attention is directed once more to the lack of necessary parallelism between blood cH on the one side and probable internal acidity and receptor activity on the other (cf. Bernthal, 1938). In the present experiments, of the three experimental fluids the "asphyxial" must have had an intermediate cH, yet its influence on receptor activity and probably cell cH was greatest. Until this application of the "reaction" theory (Gesell, 1925, 1929) to the chemoreceptors fails to meet its requirements, we may ascribe the addition of hypoxic and hypercapnic excitation at the carotid body to changes associated with convergence of their respective acid mechanisms probably upon common receptor cells (Winder, 1937b; von Euler, Liljestrand and Zotterman, 1939).

The present data extend those of Bernthal (1938), von Euler *et al.* (1939), Gesell *et al.* (1940) and others, which indicate that *tonic* activity of the carotid

bodies (in anesthetized animals) during eupnea is contributed to by the combined effects of resting  $O_2$  and  $CO_2$  tensions in the blood. They indicate that in the combination of hypoxic and hypercapnic respiratory effects in the organism as a whole, one must now consider *first*, direct combination at the carotid body; *second*, a probable direct combination centrally as affirmed by Gellhorn and Lambert (1939) and allowed by the data though not the conclusion of Dumke *et al.* (1941); and *third*, indirect combination through interaction at the "center" of the *pre-combined* reflexogenic and centrogenic influences (cf. Gesell *et al.*, 1940). It must be recognized that particularly in the more intricate nervous centers, the local combination includes potential depression concurrent with potential excitation (Gesell, 1939; Winder and Winder, 1941; Moyer and Beecher, 1941).

#### SUMMARY

The isolated carotid body regions of anesthetized dogs were perfused by means of a continuous artificial circuit with continuous gaseous equilibration of the basal perfusion fluid (heparinized blood and Locke's solution). Brief substitutions of experimental fluids equilibrated with gases high in  $CO_2$ , low in  $O_2$ , or a combination of the two, yielded reflex respiratory responses such that the combination effect on minute-volume of  $CO_2$  and low  $O_2$  was approximately equal to the sum of their individual separate effects.

The results are interpreted in terms of the theory that intracellular cH is a factor in the control of chemoreceptor activity.

The chemoreceptors are considered as one of several probable sites for mutual facilitation of hypoxic and hypercapnic stimulation of respiration.

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# THE FLOW OF LYMPH FROM THE LUNGS OF THE DOG

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If one uses the conventional reasoning relative to exchanges between the blood and tissue fluids, the lungs of mammals apparently have little need for a system of lymphatic vessels. The pressure in the pulmonary capillaries has never been measured directly, but it should be low in view of the extent of the capillary bed and the low pressure in the pulmonary artery. At the same time the blood in the lungs has the colloid osmotic pressure of the blood throughout the body, so that a relatively dry tissue should result. In the face of these considerations, which seem to make an extensive system of lung lymphatics unnecessary unless under the stress of pulmonary infection or cardiac incompetence, there stands the fact that the lungs are profusely provided with lymphatics.

Cunningham (1916) showed that the capillary lymphatics of the mammalian lung do not extend beyond the alveolar atria and Miller (1937) found no lymphatics beyond the alveolar ducts. These findings mean that proteinized fluid in the alveolar walls or actually within the alveoli does not enter the lymphatic system until the beginning of the respiratory part of the lung lobule is reached. The lymphatics of the lungs can be divided into a superficial and a deep set. The former is situated in the pulmonary pleura; the latter accompanies the bronchi, the pulmonary arteries, and the pulmonary veins. It also forms a dense network in the connective tissue septa between the secondary lobules. The two sets of lymphatics are in communication within the pleura and at the hilum of the lung. The pleural lymphatics eventually unite to form trunks which, together with the lymphatics coming from the interior of the lung along the pulmonary veins, enter the tracheobronchial lymph nodes (Miller, 1937). Wherever lymph is formed it flows eventually to nodes at the root of the lung or along the trachea.

Sappey (1874) declared that in mammals most of the lymph from the right lung entered the venous system through the right lymphatic duct, whereas the thoracic duct received lymph from the left lung. Variations on the left side were common and occasionally a vessel entered the junction of the jugular and subclavian veins quite independently of the thoracic duct.

In man, Rouvière (1932) reported that lymph from the superior and upper middle part of the left upper lobe entered the left tracheobronchial chain of nodes and lymphatics to reach the thoracic duct or the junction of the left subclavian and left jugular veins. The rest of the lymph from the left lung, together with some from the lower right lobes enters the nodes at the bifurcation

of the trachea. Efferent lymphatics from this group of nodes practically always pass to the right tracheobronchial nodes, rarely to the left. These nodes also receive lymph from the upper lobes of the right lung. The major part of the lung lymph enters the venous system at the junction of the right jugular and right subclavian veins, either directly or in conjunction with the right lymphatic duct.

In the dog Baum (1918) found that lymph from both lower lobes entered the medial tracheobronchial lymph node. His efforts to trace the lymph flow from other lobes and the possible communications between the right and left lung lymphatics either directly or through nodes are not clear.

In 1931, being under the impression that lymph from the heart and lungs entered the thoracic duct, we made Starling heart-lung preparations in dogs, cannulated the duct at the entrance into the left subclavian vein and ligated it just above the diaphragm. Under these circumstances we believed that all the lymph which might be obtained from the thoracic duct must come from the heart and lungs. No effort was made to cannulate or even observe the right lymphatic duct. In experiments—wholly satisfactory from a technical point of view—no lymph or, at the best, a few drops were collected from the thoracic duct. Either little or no lymph was formed in the chest or another path carried it to the blood.

Later, through a posterolateral incision, lymphatics were cannulated at the root of the right lung. These vessels were filled with lymph but it was always grossly bloody, due undoubtedly to lung trauma incident upon the method of exposure. The experiments did show that lymph flowed freely from the lungs if they had been rendered abnormal.

Lorber (1940) used a Starling heart-lung preparation in dogs, again with the thoracic duct tied just above the diaphragm and cannulated at the entrance into the subclavian vein. In accord with our experience he found no increase in lymph flow in spite of the development of massive pulmonary edema.

During a long period of work upon the flow and composition of lymph from the heart of the dog (Drinker et al., 1941), several lymphatics were seen connected with a lymph node under the superior vena cava and along the right side of the trachea. This node is also in the line of cardiac lymph flow, but with experience, particularly that gained from injections into the heart muscle, cardiac vessels can be distinguished from the remaining lymphatics entering the node which carry lymph from the lungs. The facts relative to the lymph collected, with proof as to lines of drainage from the lungs, form the substance of this paper.

**EXPERIMENTAL PROCEDURE AND RESULTS.** Healthy, young, adult dogs of medium size, anesthetized with nembutal (40 mgm. per kgm. intravenously) were used. Several cats were examined but the lymphatics were too small to cannulate successfully. It is impossible to reach the lung lymphatics without opening the chest, so that the trachea was cannulated and artificial respiration given by a pump delivering constant inspiratory volumes of air or oxygen with expiration following passively—that is, without suction.

The thorax was opened by removal of a section of sternum plus 3 to 5 ribs,

depending upon the extent of the exposure required. The ribs were cut about 3 cm. from their sternal connections. Both mammary arteries were tied and divided, and the thymus was removed if present in any size. Beneath the superior vena cava, on the right side of the trachea, 5 to 7 cm. from the bifurcation, one finds the tracheobronchial lymph node which receives lymph from both the heart and lungs. This node was pulled gently upward and to the right and a vessel entering upon the lower side was cleaned and isolated. It is invariably the largest of several entering the node from the lungs. This vessel was tied and cannulated with a small pyrex glass cannula. Care was taken to support the cannula in such a way that the tip lay in the line of normal drainage. The lymphatic selected is larger than the cardiac lymphatic and, being further from the heart, the motion at the point of cannulation was not so disturbing. A fine wire with a small loop at the end, dipped in dry heparin, was placed in the cannula to prevent the lymph flowing into it from clotting. The vessel cannulated is not the entire pathway for lymph from the lungs. Other and variably placed lymphatics exist and the results gained from this single trunk are thus indicative of general trends but do not give quantitative results for the total pulmonary lymph flow.

Early in the experiment, 20 cc. of Ringer's solution per kilogram of body weight were given intravenously to insure a sufficient amount of fluid in the tissues. Systemic blood pressure was taken from the femoral artery with a mercury manometer. Pulmonary arterial pressures were taken with a second mercury manometer connected to a stilette cannula in the pulmonary artery (Swift et al., 1922).

It is probable that lymph flow in the unopened chest is somewhat greater than that obtained in our experiments. The preparation, admitting the disadvantage of artificial respiration is, however, far less abnormal than the usual open chest experiment, in that the exposure through the anterior mediastinum is so small as to permit one to see only the tips of the lungs and the base of the heart within the pericardium. There is thus little drying, etc., factors so prominent in most open-chest experiments and obviously detrimental in an effort to secure lymph from normal lungs. Rate of flow was measured by collecting lymph for a given period of time into weighed tubes which were then reweighed. Protein determinations were made by means of the Zeiss dipping refractometer calibrated against known samples of lymph.

A. *The composition of lung lymph.* Table 1 shows the protein concentration, in per cent, of lung lymph collected from 18 dogs. The average amount of protein was 3.66 per cent, with a range from 2.81 to 4.65 per cent. In protein content, lung lymph is very much like cardiac lymph. It contains albumin and globulin in the ratio found in blood and in lymph from other regions. It also contains fibrinogen and clots. Erythrocyte and leucocyte counts are included in table 1. The relatively large number of red cells in the lymph from the early experiments is ascribable to slower and less efficient operating. It became less as technique improved. The white cells, practically all lymphocytes, are due to the passage of the lymph through several nodes before reaching the point of cannulation.



B. *Conditions affecting the flow of lung lymph.* 1. *The normal flow of lung lymph.* Figure 1 is a typical experiment showing the normal flow of lung lymph from a single vessel over a period of several hours. Lymph from the lungs, which are in constant motion, flows steadily under normal conditions. Table 1 shows the average lymph flow in a series of 18 dogs. This, as has been pointed out, does not represent the total amount of lymph produced by the lungs, but the amount obtained from one of several efferent lung lymphatics. The average amount of lymph from this single lymphatic was 17.9 mgm. per minute, with a wide range of from 3.7 to 59.4 mgm. per minute.

2. *Increased pulmonary ventilation.* Artificial respiration was provided by a pump calibrated to deliver a given amount of air in 17 inspirations per minute.

TABLE 1  
*Protein concentration, lymph flow, and cell content of lung lymph from 18 dogs*

NUMBER OF EXPERIMENT	PROTEIN	AVERAGE LYMPH FLOW PER MINUTE	ERYTHROCYTES PER CM.	LEUCOCYTES (LYMPHO- CYTES) PER CM.
	<i>per cent</i>	<i>mgm.</i>		
1	3.38	23.1		
2	3.09	14.6	22,350	23,850
3	3.71	22.7	13,850	11,900
4	3.87	6.8	20,100	8,850
5	3.77	6.5		
6	4.00	3.7		
7	3.76	3.9	6,800	2,900
8	4.10	5.1	30,700	28,100
9	3.05	27.8	17,900	16,300
10	4.00	4.6	100	100
11	3.63	11.2	15,800	15,000
12	3.75	7.1	7,600	20,500
13	3.48	4.5	900	22,800
14	2.81	10.3	3,900	44,600
15	3.26	37.7	200	26,600
16	3.08	59.4	1,500	13,700
17	4.46	41.9	300	76,400
18	4.65	30.6	0	21,500
Average.....	3.66	17.9	9,533	22,206

As soon as the chest was opened the pump was so adjusted as to provide adequate ventilation, usually from 2.5 to 3.25 liters per minute, depending upon the size of the animal. Figure 2 shows one of a group of experiments in which, during the control period, while lymph was being collected, the animal was receiving 2.5 liters of air per minute. Between the vertical lines 1 and 3, pulmonary ventilation was increased to 4.3 liters per minute. The lymph flow immediately decreased and remained constant until the ventilation was reduced to its original volume. The flow then rose again to its control level.

The fall in lymph flow with increased artificial respiration is probably not what occurs when there is a normal increase in breathing. There is evidence that heightened negative pressure in the thorax causes transudation into the

pleural sacs. Graham (1921) held that in the presence of pulmonary edema, deep respiratory movements, with consequent increased degrees of negative intrathoracic pressure, sucked fluid from the lungs into the pleural sacs. Brock and Blair (1931), using a heart-lung preparation, showed the development of pleural transudates just as Graham had postulated. Yamada (1933) made pleural punctures in several hundred healthy soldiers. He obtained pleural fluid in 29 per cent of his subjects who had been entirely quiet. Seventy per cent of the same subjects yielded fluid after a period of severe work. This means that negative pressures of 30 to 40 mm. Hg, such as occur readily during work when inspiration is full, with a return to 6 mm. or thereabouts at the end of expiration, cause fluid to leak from the surface of the lungs. The effect of the change in pressure on the volume of the lung capillaries is thought to be slight. But if a transudate can be made to accumulate in the pleural sacs as a result of

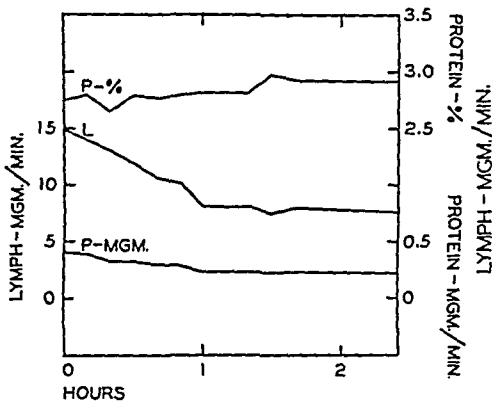


Fig. 1

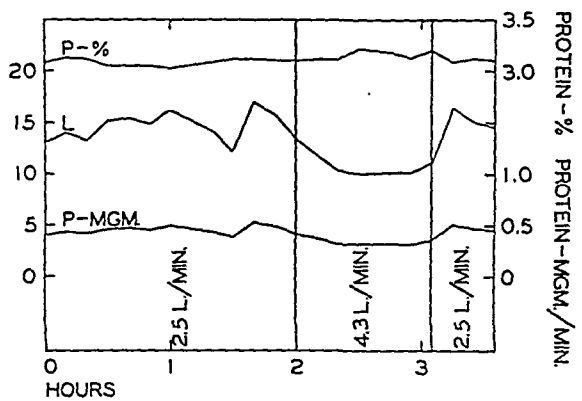


Fig. 2

Fig. 1. Normal flow and composition of lung lymph. *Upper curve*, protein in lung lymph in per cent. *Middle curve*, milligrams of lymph per minute. *Lower curve*, lymph protein in milligrams per minute. *Ordinates*, as designated. *Abscissae*, time in hours.

Fig. 2. Effect of over-ventilation. *Upper curve*, protein in lung lymph in per cent. *Middle curve*, milligrams of lymph per minute. *Lower curve*, lymph protein in milligrams per minute. *Ordinates*, as designated. *Abscissae*, time in hours.

hard exercise, it would seem that accumulation of fluid in the lung tissue must occur at the same time and that the high negative pressure at full inspiration may be part of the cause. That the lymphatics would take part in the removal of this fluid is reasonably certain.

With the chest open and with artificial respiration, pressure upon the lung capillaries is always positive and when respiration is increased by altering the stroke of the respiration pump, but not the rate—as was done in these experiments—a relatively high positive pressure will be present through most of the respiratory cycle. The result will be the maintenance of tissue pressure in the lungs upon a positive level rather than the customary negative pressure and a tendency to reduce transudation of fluid rather than to increase it.

3. *Continuous intratracheal insufflation.* After a control period, during which artificial respiration with pure oxygen was given by means of the respiratory

pump, the animal was shifted to intratracheal insufflation with pure oxygen, lung inflation being held just below the complete inspiratory position. Figure 3 shows a typical experiment in this series. With no lung movement at all—arrows 1 and 2—and only the massage caused by the movements of the heart and pulmonary vessels, the flow of lymph fell abruptly, but did not stop entirely. As soon as the intratracheal tube was removed and artificial respiration again supplied by the pump, the flow of lymph returned to approximately the control level. In this experiment, cessation of lung movement, with the lungs not ab-

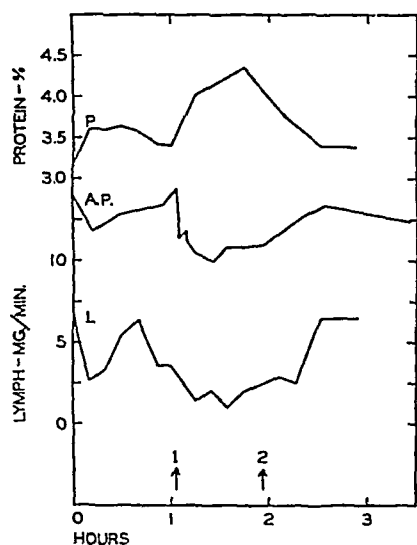


Fig. 3

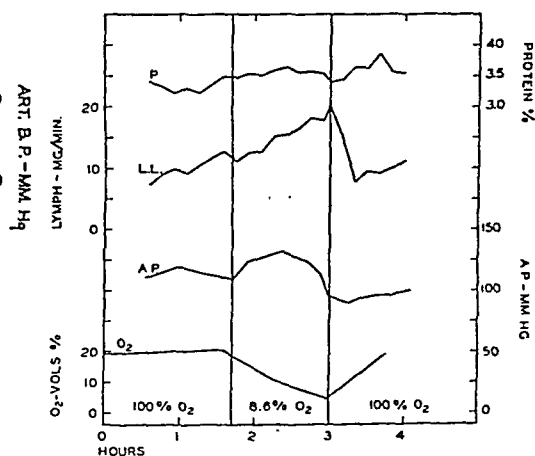


Fig. 4

Fig. 3. Effect of holding lungs motionless. *Upper curve*, protein in lung lymph in per cent. *Middle curve*, pressure in the femoral artery in millimeters of mercury. *Lower curve*, milligrams of lymph per minute. *Ordinates*, as designated. *Abscissae*, time in hours. Between arrows 1 and 2 rhythmical artificial respiration ceased and continuous intratracheal insufflation was employed. At arrow 2 rhythmical artificial respiration was again initiated.

Fig. 4. Effect of lowering alveolar oxygen. *Upper curve*, *P*, protein in lung lymph in per cent. *Second curve*, *L.L.*, milligrams of lymph per minute. *Third curve*, *A.P.*, pressure in the femoral artery in millimeters of mercury. *Fourth and lowest curve*, *O<sub>2</sub>*, cubic centimeters of oxygen per 100 cc. of arterial blood. *Ordinates*, as designated. *Abscissae*, time in hours. Between the vertical lines the animal was given 8.6 per cent *O<sub>2</sub>* and 91.4 per cent N instead of 100 per cent oxygen. Rate and stroke of respiratory pump constant throughout the experiment.

normally distended, resulted in a fall of systemic blood pressure but not to a serious degree and certainly not enough to change the pulmonary blood pressure significantly. Lymph flow was less than in the moving lung and reached a high figure after rhythmical artificial respiration was restored. The degree to which continuously insufflated oxygen reaches all parts of the lungs is problematical and the possible concomitant effects of oxygen lack in parts of the lungs can not be excluded. But even if abnormal amounts of tissue fluid or lymph have been formed during the period of quiescence, it is apparent that respiratory movements are important for causing lymph flow.

4. *Reduced oxygen.* Figure 4 shows one of a series of experiments in which, after a control period of ventilation with 100 per cent oxygen, the animal was given a mixture of 8.6 per cent oxygen and 91.4 per cent nitrogen by the respiration pump for 73 minutes. When inspiratory movements which often accompany inadequate oxygenation occurred they were controlled by intravenous injections of 1 cc. of 1 per cent curare, diluted to 10 cc. with Ringer's solution. Femoral arterial pressures were recorded throughout and the oxygen content of the arterial blood was determined frequently. As soon as the animal began to receive the low oxygen mixture, the flow of lymph from the lungs increased. The femoral blood pressure fell after the initial rise which sometimes attends abrupt anoxemia. When the arterial blood pressure fell to about 95 mm. of mercury and the oxygen saturation of the arterial blood was 4.42 volumes per cent, the ventilation of the animal was shifted back to pure oxygen. Lymph flow returned almost immediately to the normal level. That the period of severe oxygen lack did no permanent damage to the blood capillaries is shown by the fact that at the end of the experiment, when the oxygen content of the blood had

TABLE 2

*Oxygen and carbon dioxide in the arterial blood during ventilation with 100 per cent oxygen, low oxygen, and return to the original condition*

TIME	OXYGEN PER 100 CC. BLOOD	CARBON DIOXIDE PER 100 CC. BLOOD	REMARKS
<i>minutes</i>	<i>cc.</i>	<i>cc.</i>	
0	19.31	29.44	Changed from pure oxygen to
95	20.58	28.95	8.6 oxygen at 107 minutes.
135	10.86	31.7	Returned to 100 per cent
178	4.42	14.5	oxygen at 180 minutes
225	19.04	29.98	

returned to the control level of 19 vols. per cent, there was no increase in the amount of lymph and no appreciable increase in protein content.

Table 2 gives the oxygen and carbon dioxide content in cubic centimeters per 100 cc. of blood during the experiment. In this animal blood oxygen was at a normal level whenever 100 per cent oxygen was used for ventilation, but the carbon dioxide was abnormally low throughout the entire experiment and fell markedly during the period when 8.6 per cent oxygen was supplied. In other experiments where the carbon dioxide content of the arterial blood was 39 to 43 cc. and the oxygen content fell markedly on ventilation with low oxygen, lymph flow increased as in this case, so that low oxygen alone causes an increase in the permeability of the lung capillaries. The relation of this effect to the carbon dioxide content and combining power of the blood, particularly to the situation provided by holding the blood carbon dioxide above normal during ventilation with low oxygen mixtures are not known. Maurer (1941) has shown that in the presence of a normal oxygen content, increased carbon dioxide in the blood caused the capillaries of the heart and of the skin, mucous membranes,

etc., in the head of the dog to become more permeable as judged by the outflow of lymph, but the result was never as marked as that obtained from uncomplicated anoxemia.

5. *Increased pulmonary blood pressure.* The effect of increasing pulmonary blood pressure on the flow of lymph from the lungs was observed in a number of dogs. Femoral arterial and pulmonary arterial pressures were recorded with mercury manometers. A stilette cannula, as described by Swift, Haggart and Drinker (1922), which is thrust through the wall of the pulmonary artery, was used for the pulmonary blood pressure measurements since it causes no obstruction to pulmonary blood flow and can be inserted without manipulation of the pulmonary vessels. After the pericardium was incised longitudinally and stitched to the sides of the opening in the chest, a ligature was passed around the pulmonary veins and attached to a screw clamp which could be so adjusted as to produce and maintain any desired degree of vein compression (Drinker, Peabody

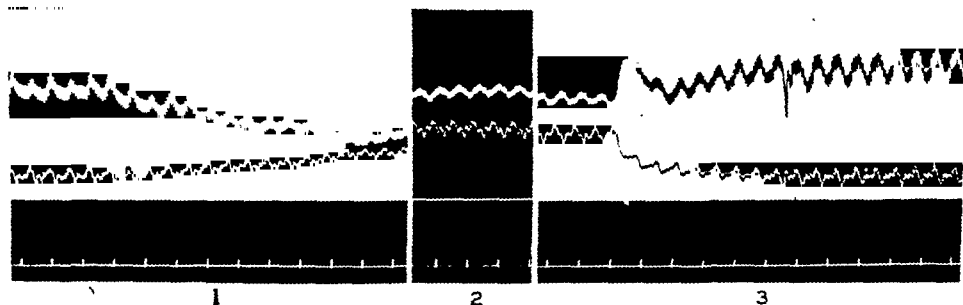


Fig. 5. Effect of compressing the pulmonary veins. Tracings of systemic (*upper curve*) and pulmonary artery (*lower curve*) pressures during 1, tightening; 2, maintenance of steady compression, and 3, release of the compression clamp on the pulmonary veins. *Straight line*, base for pulmonary blood pressure; *bottom line*, time in 6-second intervals and base for systemic pressure. Tightening of the compression clamp began during tracing 1, was held steady as shown in tracing 2, and was released in the early part of tracing 3.

and Blumgart, 1922). This arrangement obstructs the flow of blood in the pulmonary veins just as they reach the left ventricle, but does not affect other vessels entering and leaving the heart. In order to put the clamp in place, a longer opening must be made in the chest, but the exposure required is essentially at the base of the heart and even in these experiments was less extensive than in many direct examinations of the heart and lungs.

Lymph flow was measured during a control period. The pulmonary arterial cannula was then inserted and connected to the manometer and, while recording pulmonary and systemic pressures, the pulmonary veins were compressed by tightening the clamp. Figure 5 shows kymographic records of the systemic and pulmonary artery pressures in a typical experiment. The upper curve is that of pressure in the femoral artery, that just beneath it of pressure in the pulmonary artery. Tightening of the compression clamp upon the pulmonary veins began during segment 1 of the tracing, and caused a simultaneous fall in systemic and rise in pulmonary artery pressures. The clamp was closed slowly until a point

was reached where systemic and pulmonary arterial pressures were stabilized at new levels, as shown in segment 2 of the tracing. The sequence of events in such an experiment is as follows: As the pulmonary veins were slowly compressed, blood flowed steadily into the right side of the heart from the cavae and was pumped into the pulmonary circulation where, on account of obstructed flow, it formed an enlarging pool. After a time, venous occlusion being moderate in extent, the pulmonary vascular bed was stuffed full and blood began to move over into the left side of the heart in fair volume so that cardiac output improved and systemic blood pressure rose. At the same time, pressure in the pulmonary artery, due to a competent right ventricle, was held at a high level. In segment 3 of the tracing, the double mark upon the base line for the pulmonary arterial pressure signalled the point at which the pulmonary vein clamp was abruptly released. There was an immediate rise in the systemic blood pressure as the blood imprisoned in the lungs flowed rapidly into the left side of the heart. The pulmonary arterial pressure fell to the original level.

That part of the experiment seen in segment 2 of the tracing is probably a very good expression of what is going on in a case of mitral stenosis, where there is a fair degree of cardiac competence but decompensation is close at hand. Under such circumstances the lung tissue is overfilled with blood; the alveoli are encroached upon by the swollen capillaries; lung movements are reduced since the highly elastic lung has been converted into a sort of erectile tissue.

Two fundamental effects may be expected from obstruction of the pulmonary veins; first, increased pressure in the lung capillaries, and second, decreased oxygenation of the blood due to interference with alveolar ventilation. Both of these favor leakage of plasma from the alveolar capillaries. The lung edema will mean increased tissue fluid in the alveolar walls with the possibility of leakage through the alveolar epithelium into the alveolar air spaces.

Figure 6 is a more complete representation of what occurred in the experiment under discussion and to a degree shown in figure 5. Lung lymph was collected for 3 hours and the amounts in milligrams per minute varied but little as seen in the second curve, *L.L.* At the beginning of the third hour the pulmonary veins were compressed for 10 minutes and then released. The effects of this obstruction upon systemic and pulmonary blood pressures are seen in figure 5, and are shown diagrammatically in figure 6, curve 3, *A.P.*, and curve 4, *P.A.P.*

In this animal, the pulmonary venous obstruction caused a sharp rise in lymph flow which, during the following hour and a quarter, fell to normal. With the increase in volume there went a fall in the per cent of protein in the lymph, which occurred in spite of the fact that in such experiments the increased pressure in the pulmonary capillaries invariably caused the lymph to appear bloody, but in the lungs as in other regions, escape of red cells from the capillaries frequently cannot be correlated with escape of plasma. For example, if the veins to a part, such as the foot of the dog, are slightly obstructed and the animal walks at a steady rate during the experiment, lymph flow from the foot becomes greater as the capillary pressure is raised and red cells, which during periods of normal venous pressure have been difficult to find in the lymph, soon become very

numerous. But with this increase in the apparent blood content of the lymph, the percentage of protein in it usually falls.

In the experiment described, a certain degree of anoxia accompanied the vascular congestion and possibly contributed a direct increase in the permeability of the capillary endothelium which made the heightened pressure in the pulmonary circuit even more effective in forcing fluid out of the capillaries and thus bringing about increased production and flow of lymph. In this case arterial oxygen was 18.2 cc. per 100 cc. of blood prior to vein compression and fell to 14.7 cc. per 100

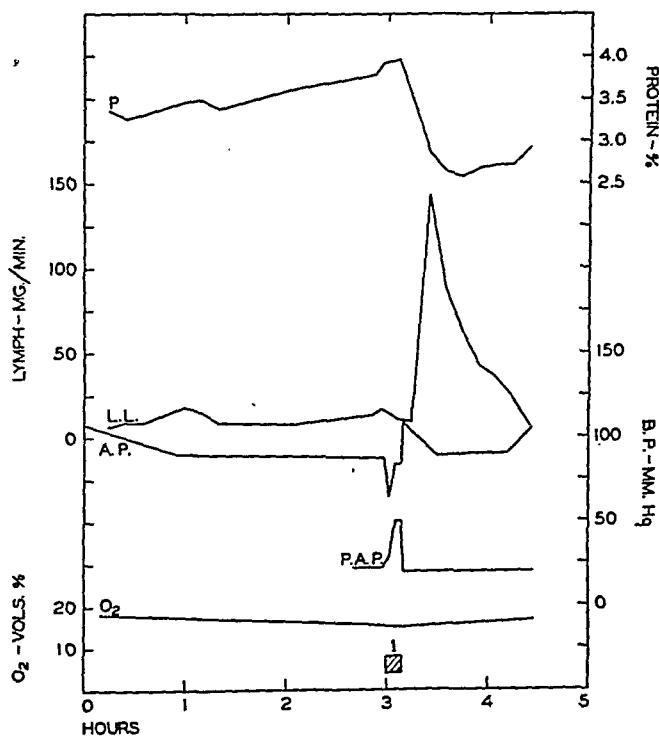


Fig. 6. Analysis of the effects of pulmonary vein compression. *Upper curve, P*, protein in lung lymph in per cent. *Second curve, L.L.*, milligrams of lymph per minute. *Third curve, A.P.*, pressure in the femoral artery in millimeters of mercury. *Fourth curve, P.A.P.*, pressure in the pulmonary artery in millimeters of mercury. *Fifth curve, O<sub>2</sub>*, cubic centimeters of oxygen per 100 cc. of arterial blood. *Ordinates*, as designated. *Abscissae*, time in hours. During the period measured by the hatched square, 1, the pulmonary veins were compressed.

cc. just before releasing the clamp and was 16.5 cc. at the close of the experiment when lymph flow had fallen to normal. This means definite but not pronounced oxygen lack. Corresponding figures for arterial carbon dioxide were 39.5, 38.3 and 39.7 cc. per 100 cc. of blood. These figures are quite normal and point to increased capillary pressure as the chief, if not the single cause of the augmented lymph flow. In another experiment, which resulted in an even larger flow of very bloody lymph, blood oxygen was 20.3 cc. per 100 cc. prior to pulmonary congestion, 17.9 cc. during the period when clamping was maintained, with a rise to 19.1 cc. 64 minutes after release. Figures for carbon dioxide content of the

same samples were 39.5, 35.3, 40.9 cc. per 100 cc. In this case lymph flow was still above the normal level when the experiment was ended and was extremely bloody.

C. *The path of drainage of lung lymph.* Using the method of exposure of the anterior mediastinum, which has been described, it is easy to clean the subclavian and adjacent veins with absolute thoroughness and thus to expose all possible entrances of the right lymphatic duct and the thoracic duct into the veins at the base of the neck. The thoracic and right ducts, or upon the right side, the efferent duct from the most cranial of the right tracheobronchial nodes were cannulated as they entered the veins.

These preparations made and lymph flow being normal, a glass catheter was passed down the trachea and into a right or left lower lobe bronchus, being pushed in gently until binding of the lower end precluded leakage. Three cubic centimeters of the dye T-1824 in physiological salt solution were then introduced into the catheter and when it was clear of fluid, so that overflow of the dye into the opposite lung would not occur, the catheter was withdrawn and artificial respiration, which had gone on unbrokenly in other parts of the lungs again involved the injected lobe. In some instances the dye was introduced in equal amounts into both lower lobes or into other lobes upon either side. The lymph from the thoracic duct and from the vessel cannulated upon the right side was watched for the first appearance of the intense blue dye and at the end of the experiment an autopsy was performed in order to follow the course of the stained lymphatics and nodes and to ascertain the distribution of the dye which had been given.

Table 3 shows the results of this series of experiments. Invariably the dye appeared first and in greater concentration on the right, regardless of where it had been placed. The medial tracheobronchial node was always deeply stained and the tracheobronchial nodes of the injected side were usually more heavily stained than those of the opposite side. In experiment 7 a trace of blue appeared in the thoracic duct two hours after its injection into the lung and an hour and a quarter after its first appearance in very concentrated amount in the right duct. Examination of mesenteric lymphatics at this time showed them to be slightly blue. The trace of dye in the thoracic duct had undoubtedly reached it, not from the lungs, but indirectly from the blood after absorption into the blood in the lungs, something that occurs in the case of T-1824, though in small amount.

In a number of experiments, usually when the dye had been placed in the left lung, either none or only a trace of dye could ever be detected in the thoracic duct. At autopsy a separate, blue-stained vessel of consequential size was sometimes found entering the junction of the jugular and subclavian veins on the left, quite independently of the thoracic duct. It has always been assumed, in spite of Sappey's work, that, by tying off the thoracic duct just above the diaphragm and cannulating it at its venous entrance, all lymph except from the heart and left lung would be excluded. The presence of this separate vessel, however, explains the fact that very often in our experience and in that of others, there is absolutely no flow from the cannulated duct under such circumstances.



It is also possible that in the dog somewhat the same situation exists as in man; i.e., that lymph from the left lower lobe, where the dye was placed, after entering the medial tracheobronchial node, which was always deeply stained, passed much more readily to the right tracheobronchial nodes than to the left. Under these circumstances the dye would naturally be found first and in greater concentration on the right, as was our invariable experience. The presence of connecting lymphatics between the nodes on the right and left of the trachea could account for the staining of the nodes and for the small amount of dye which was found later in the thoracic duct or in a separate vessel.

DISCUSSION. The experiments we have described have their chief value in the demonstration of a constant and considerable flow of lymph from the lungs, under conditions which are abnormal only in that the chest is open and artificial

TABLE 3

*The appearance of dye in the final lymphatic path to the blood after intrabronchial injection into the lungs of the dog*

NO. OF EXPERIMENT	LOCATION OF T-1824	FIRST APPEARANCE OF DYE	AMOUNT OF DYE						COMMENTS
			Right duct	Thoracic duct	Separate branch into veins on left	Medial tracheobronchial node	Right tracheobronchial nodes	Left tracheobronchial nodes	
1	Right and left lungs	Rt. duct	+++++	—	+	+++++	+++++	+++++	
2	Right and left lungs	Rt. duct	+++++	++	—	+++++	—	+++++	
3	Right lung	Rt. duct	+++++	—	—	+++++	+++++	—	
4	Right lung	Rt. duct	+++++	—	—	+++++	+++++	++	
5	Right lung	Rt. duct	+++++	—	—				Nodes not examined
6	Right lung	Rt. duct	+++++	—	++	+++++	+++++	+++++	
	Left lung	Rt. duct	+++++	±	—	+++++	+	+++++	
	Left lung	Rt. duct	+++++	±	++	+++++	+	+++++	Abdominal lymph blue
9	Left lung	Rt. + T. duct	+++++	+++	+++	+++++	+	+++++	
10	Left lung	Rt. duct	+++++	+	++	+++++	++	+++++	
11	Left lung	Rt. duct	+++++	—	++	+++++	++	+++++	

respiration by means of positive pressure, replaces normal, negative pressure breathing. Reasons have been given for the belief that in the closed chest under the usual conditions of breathing, lymph flow is probably greater than under the circumstances of our experiment.

It has been shown that overventilation by positive pressure artificial respiration reduces lymph flow and that in the absence of respiratory movements the flow is also reduced. This last observation is of practical interest in relation to the condition of affairs in a lung rendered quiescent by pneumothorax. Where lymph drainage is interrupted, fibrosis occurs and this fact may well be of importance in the healing of infected areas in the lungs.

We have been able to report the effects of ventilating the lungs with mixtures low in oxygen and have attributed the increased lymph flow, which invariably

occurred, to increased permeability of the pulmonary capillaries. Against this assertion it may be held that no data upon cardiac output have been supplied. Granting the increased flow of lymph, the question arises as to whether this is not a simple expression of a larger, better circulated capillary bed which has occurred as a result of anoxia. It is our belief that this second explanation of augmented lymph flow is not correct. The reasons for this belief, or better, conviction, are as follows:

1. During the control period when ventilation with pure oxygen was used and throughout the subsequent low-oxygen ventilation, the movements of the lungs have been absolutely the same, curare being used to prevent spontaneous respiration. The anesthetized and curarized animal made no respiratory or other movements which could increase cardiac filling and thus induce greater cardiac output.

2. There is no evidence that the pulmonary capillaries open and close as active adaptations to circulatory requirements, their changes in diameter and in conduction of blood being passively controlled by cardiac output and probably also by respiratory movements.

3. The immediate effects of anoxia upon cardiac output are not reported to increase it, even in naturally breathing subjects (Grollman, 1932; Doi, 1921).

4. If the pulmonary capillaries lose tone as a result of asphyxia and the vascular bed becomes greater, the filtering pressure in the capillaries will fall unless the output of the right ventricle is increased. Simple mechanical conditions for enhanced filtration from pulmonary capillaries have not been provided by our experiment and the conclusion seems unescapable that the pulmonary capillaries are peculiarly susceptible to oxygen lack as a cause of increased permeability.

This conclusion is of importance clinically since edema of the lungs progressively excludes capillaries from access to the alveolar air and, therefore, a condition initiated by oxygen lack possesses capacity to decrease oxygen supply and thus a vicious circle is established. The lymphatics of the lungs counteract this course of events by steady removal of proteinized fluid and if, as is common, the pulmonary edema is accompanied by some increase in breathing, it is probable that lymph flow is greater than normal.

Another point relative to the experiments upon anoxia and those in which passive congestion of the lungs was produced by compression of the pulmonary veins, is the use which may be made of the volume and composition of the lymph to indicate the progress of pulmonary edema, or of increased fluid in the lungs from inflammation. The experimental analysis of such conditions has always suffered from inability to follow progressive changes, the autopsy and the histological section being our best objective evidence of the degree of edema, particularly in the early stages.

We have been able to give little attention to the effects of adequate oxygen in checking the progress of pulmonary edema. So far as has been seen the increased permeability caused by oxygen lack occurs quickly and is rapidly reversible if the process has not gone too far. The same general results upon lymph flow accompany experiments upon the heart and the cervical region in the dog.

Since the lung capillaries not only owe their oxygen supply to the alveolar air but are so placed that abnormal permeability upon their part due to anoxia induces further anoxia, it is impossible to utilize knowledge gained from other regions in arriving even at opinions in regard to recovery of normal permeability. It is, however, clear that administration of oxygen will become progressively less efficacious as pulmonary edema progresses. There is a "factor of safety" in all physiological conditions and at times it may prove deceptive. Thus the capillaries in a region of the lung where pulmonary edema has occurred may still possess a fair measure of normal resistance to leakage, but if adequate oxygenation is not provided, the unfavorable condition of the capillary walls may cause loss of the measure of normal permeability they still possess. If oxygen administration prevents this disastrous train of events, the earlier it can be given the better. Many questions arise in regard to the use of oxygen in combating pulmonary edema. For example: Is it essential that oxygen be given continuously, or will good results be obtained by periods of, let us say, 30 minutes' administration of oxygen, then 15 minutes of air or some such combination? If dyspnea is not marked, would the lungs clear more rapidly by inducing an increase in breathing through addition of carbon dioxide and so promoting increased lymph flow? If diffuse pulmonary inflammation is present, as, for example, in phosgene poisoning, how effective is oxygen or oxygen plus carbon dioxide in combating the effect? Certainly in such states anoxia adds to the effect of the poison in causing capillary leakage, but how far oxygen may be of immediate benefit to the injured tissue is not known.

In all of these problems studies of pulmonary lymph flow and composition may be useful and experiments are now in progress to supply this information.

#### SUMMARY

1. A method for collecting lymph from the lungs of the dog is described. The experiment requires opening the chest to expose the anterior mediastinum and artificial respiration is used during its course.
2. The composition of lung lymph resembles that of cardiac lymph. In 18 dogs the average protein content was 3.66 per cent.
3. When artificial respiration is unduly great or when the lungs are quiescent lymph flow is greatly reduced.
4. Ventilation with a mixture low in oxygen invariably increases lymph flow. If pressure in the pulmonary veins is heightened, lymph flow is greatly augmented and the lymph soon resembles blood.
5. The drainage of lymph from both lungs is in the main via the right lymphatic duct, comparatively little lung lymph being delivered to the circulation by the thoracic duct.

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## ERRATA

Amer. J. Physiol. **135**: no. 1, p. 1, 1941. ADRENALINE ESTER. Derek Richter and F. C. MacIntosh

- (1) p. 2, line 19. For 1 mgm. adrenaline *read* 1  $\mu$ gm. adrenaline.
- (2) p. 3, line 1. For 1 mgm. adrenaline *read* 1  $\mu$ gm. adrenaline.
- (3) line 6. For 1 mgm. adrenaline *read* 1  $\mu$ gm. adrenaline.
- (4) line 7. For 25 mgm./ml. *read* 25  $\mu$ gm./ml.
- (5) p. 4, line 1. For 6.1 mgm. adrenaline/ml. *read* 6.1  $\mu$ gm., etc.
- (6) line 2. For 1.5 mgm. adrenaline/ml. *read* 1.5  $\mu$ gm., etc.
- (7) Fig. 1, p. 2, line 4 of legend. For 2 mgm. adrenaline *read* 2  $\mu$ gm. adrenaline.
- (8) Fig. 2, p. 3, line 2 of legend. For 25 mgm. adrenaline *read* 25  $\mu$ gm. adrenaline.

Contexts of above:

- (1) The cat showed a clear blood pressure rise with 1 mgm. adrenaline.
- (2) The nictitating membrane and intestine showed a definite response to 1 mgm. adrenaline.
- (3, 4) The response with unhydrolysed urine after taking adrenaline was only slight and corresponded to less than 1 mgm./ml. adrenaline, while solution (A) containing the hydrolysed ester gave responses corresponding to 25 mgm./ml. adrenaline.
- (5, 6) . . . the amount found after taking 10 mgm. adrenaline corresponded to 6.1 mgm./ml. in the urine collected in the first 4 hours and 1.5 mgm./ml. during the subsequent 5 hours.
- (7) E, Response to 2 mgm. adrenaline.
- (8) A, 25 mgm. adrenaline.



## ERRATA

VOLUME 136: No. 1, p. 207, 1941. M. F. Warren and C. K. Drinker, The Flow of Lymph from the Lungs of the Dog.

*P. 208*, change (Drinker et al., 1941) to (Drinker et al., 1940).

*P. 213*, change Maurer (1941) to Maurer (1940).

*P. 221*, insert the following references in the bibliography:

DRINKER, C. K., M. F. WARREN, F. W. MAURER AND J. D. MCCARRELL. This Journal **130**: 43, 1940.

MAURER, F. W. This Journal **131**: 331, 1940.





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## CILIARY MOVEMENT AND CIRCULATION OF CEREBROSPINAL FLUID WITHIN BRAIN VENTRICLES IN LARVAL AND ADULT ANURANS

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In 1941 Wang and Lu reported that, when the living brain of a frog tadpole is examined under the microscope, dark particles are frequently seen darting to and fro in the brain ventricles. The movements of such dark particles offer an opportunity for studying the factors controlling the circulation of the cerebrospinal fluid. Therefore, it has been proposed to investigate *first*, the origin and fate of the dark particles, and *second*, the cause of their movements. Special attention has been paid to the second problem. Its solution has indicated that motion of the cilia on the ependymal cells covering the choroid plexus and the brain ventricles generates local currents in the cerebrospinal fluid and thereby plays a rôle in its circulation inside the ventricles. Observation has been extended to frogs, water toads and true toads, and similar results have been obtained. That the ependymal cilia play a part in the circulation of the cerebrospinal fluid hitherto has not been suspected. The results of our work here reported serve to call attention to this long neglected factor.

**METHODS.** Observations were made mainly on the tadpoles and adults of *Microhyla peulchra*. This species of anura was chosen for study because an abundant supply of its eggs and adults is found in the ponds near the laboratory of this Institute, and particularly because the body of its larva remains translucent almost up to the time of metamorphosis. Pigments in the skin of older larvae may obscure the contents of the brain case; but this is easily remedied by removing the skin before observation. For comparison, tadpoles and adults of *Rana limncharis*, *Polypedates leucomystax* and one unidentified species of toad also were used.

Young larvae under observation were laid on a thin layer of moistened cotton wool on a glass slide; older ones were placed in a small water chamber built with

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wax on the slide. For studying the cause of the movements of black particles; blood of the larva diluted with amphibian saline solution was introduced into the brain ventricles. This was done by means of a micropipette operated by a simple home-made micromanipulator, in order to avoid the escape of the cerebrospinal fluid from the brain case. In one experiment, the ectodermal anlage of hypophysis was extirpated by sucking it out at the gill-slit stage.

**RESULTS.** A. *Observation on larvae.* 1. *The origin and fate of the particle.* Black particles in the brain ventricles of anuran larvae could be easily removed by first puncturing the skull over the caudal hindbrain, and then applying gentle pressure on the skull to force the particles to come out through the punctured hole. The removed particles were either dissected into small pieces or smeared on a slide. When these preparations were examined under the microscope at a magnification of 575 diameters, no cellular structures could be discerned. The particles simply consisted of black pigmentary granules. No sign of their regeneration was found up to 27 days after their removal.

For the purpose of tracing the course of development of the particles from their first appearance to their final disappearance, tadpoles of *Microhyla peulchra* were hatched and reared in the laboratory; and dissections were made either on freshly sacrificed larvae or on specimens fixed in Destin's solution from the neurula stage to several days after metamorphosis. Just after the neural tube was completely closed, its inner wall was found to be still covered by a layer of pigmented ectodermal cells, and no dark particles appeared. Shortly after this, dark particles made their first appearance, and they increased in number *pari passu* with the gradual disappearance of pigmented cells on the inner wall of the neural tube. With larvae approximately 4 mm. long, the pigmented cells on the inner wall almost all vanished; the number of dark particles reached its climax. Usually more than 20 particles were found in the third and fourth ventricle, some moving freely in the cerebrospinal fluid, while the rest still adhered to the ventricular wall. Gradually all particles became detached and free-moving bodies. Hereafter, they decreased in number but increased in size. Small particles seemed to coalesce with each other into a bigger one. Large particles usually lodged somewhere in the caudal part of the hindbrain; they were too heavy to be moved by the currents in the cerebrospinal fluid. One to three days after metamorphosis, large particles could still be found in the hindbrain ventricle. However, they were never encountered in the brain ventricles of adult forms of *Microhyla peulchra* and other species of anura examined.

This course of development was not affected either by hatching and rearing the larva in a dark room, or by removing the ectodermal anlage of the hypophysis at the gill-slit stage.

All these facts strongly suggested that the dark particles had their origin in the pigment of the ectodermal cells covering the ventricular wall. In several series of serial sections of the entire larva of *Rana limnocharis* prepared for another purpose, we found globules of dark pigment floating in the ventricle, adhering to the surface of the ependyma and lying between cells. These findings

gave support to our conclusion. Furthermore, an opportunity to test it was found in the eggs of *Polypedates leucomystax*.

The eggs of this species of tree frog are white in color. After hatching, the larvae also are devoid of pigmentation. A few pigmented cells appear on the dorsal surface of the body, when the tadpole grows to a body-length of 6 mm. Dissections were made at different developmental stages on tadpoles hatched and reared in the laboratory. In the brain ventricles of larvae less than 6 mm. long, no dark particles were ever encountered. They first appeared after the dorsal surface of the body became scantily pigmented. But their number (3 or 4) was 5 to 7 times less than the maximum found in the larvae of the *Microhyla*. At a body-length of approximately 12 mm., the 3 or 4 particles were reduced to 1 or 2. Dark particles completely disappeared in tadpoles longer than 14 mm.

The data presented in the preceding paragraphs justify the following conclusion: the dark particles found in the brain ventricles of anuran tadpoles have their origin in the pigment of the epithelial cells covering the inner wall of the neural tube, and are gradually absorbed after the small particles have coalesced with each other into bigger ones.

2. *The cause of its movements.* When a living *Microhyla* tadpole 5 mm. long was examined under the microscope, several small dark particles were usually seen moving actively in the third and fourth ventricles of the brain. The motion of each particle consisted of both rotation and translation in a straight or curvilinear path. It darted to and fro in the ventricles, or circled around as if in a whirlpool. But both the rhythm and the path of its movements were highly irregular. With larger older larvae, 1 or 2 moving particles were observed. As the size of the particle reached the upper limit (approximately  $2.5 \times 10^{-4}$  mm., as determined by measurements of removed particles), it ceased to move, and was usually anchored somewhere in the caudal half of the hindbrain ventricle. Medium-sized particles frequently were caught in the corners of the ventricles during their motion.

The movements of dark particles were influenced neither by the exclusion of circulation nor by changes in the position of the body in space. Exclusion of circulation by decapitation rendered bloodless the vessels in the head and thereby gave a clearer field for observation. No change in the motion of dark particles was noticed, except that their movements progressively grew weaker as the preparation slowly deteriorated. With the microscope in the horizontal position, a small tadpole placed on a thin layer of cotton wool on a slide could be rotated on the dorsiventral axis of its body into any position in a vertical plane. Such change of position in space was found to have no observable effect on the motion of particles in the brain ventricles.

A convenient means for investigating the cause of particle movements was provided by the following accidental observation. In the attempt at removing the particles, some blood vessels of the skull were always severed, when the skull was punctured. Red blood corpuscles escaped into the ventricles, and showed similar movements to those of the dark particles. Then, blood from large tad-

poles or adults diluted with amphibian saline solution was deliberately injected into the fourth ventricle of the larva under observation; and the injected red cells moved in a highly similar manner as the particles originally in the ventricle. This gave us a method for direct inspection of the cause of particle movements.

When a few drops of diluted blood were introduced into the cerebrospinal fluid in the exposed fourth ventricle, the added red blood corpuscles showed the same movements as in the intact ventricle. As soon as the cerebrospinal fluid was drained away, the red cells rested motionless on the ependyma. This piece of evidence clearly indicates that the movements of the particles in the ventricles are due to local currents in the cerebrospinal fluid.

In order to demonstrate how such local currents are generated, pieces of the ventricular wall were removed and examined under the microscope. When a piece of the ventricular wall placed in a bath of diluted blood was observed under the low power (magnification: *circa* 40 diameters), red cells were seen moving near the ependymal surface. These movements were similar to those of red blood corpuscles near the ciliated surface of a piece of esophagus removed from frog and examined in the same manner. Since it is known that the ependymal epithelium also possesses cilia on its free surface, it seemed reasonable to conclude that the local currents near the ependyma were due to ciliary movements. However, the attempt at direct inspection of ciliary movement on the ependyma under high power ( $\times 575$  diameters) entirely failed. Another trial to observe the movements of ependymal cilia in the reflected light with the 'ultrapaque' microscope was also unsuccessful. The mass of nervous tissue adhering to the ependyma interfered with observation in both transmitted and reflected light; and no method was found to free the ependyma from nervous tissue without damaging the former.

The ependyma on the roof of the fourth ventricle also has cilia, and forms a thin sheet of cells suitable for microscopic examination. Placing a piece of the roof membrane stained with methylene blue and moistened with diluted blood under high power ( $\times 575$  diameters), we could observe the red blood corpuscles following the movements of the ependymal cilia. This was most clearly observed when the preparation was dying and the ciliary movements became slow.

Hence, the cause of the movements of dark particles in the brain ventricles is found in the local currents generated by the motion of ependymal cilia in the cerebrospinal fluid.

**B. Observations on adults.** Two series of observations were made on the adults of *Microhyla peulchra*, *Rana limnocharis*, *Polypedates leucomystax* and one unidentified species of *Bufo*. In one series, the movements of added red blood corpuscles in the exposed third and fourth ventricle of the removed brain were observed, whereas in the other we watched under the microscope at a magnification of 575 diameters the motion of the cilia on a piece of the roof of the fourth ventricle removed from the brain and stained with methylene blue. The results of both series were similar to the findings obtained with identical methods on the larva.

**DISCUSSION.** The results presented in the preceding section definitely

demonstrate that movements of the cilia on the ependymal lining of ventricles play a rôle in the circulation of the cerebrospinal fluid inside the ventricles, at least in amphibians. When we take into consideration the enormous number of these cilia, we must concede that their rôle in the motion of the cerebrospinal fluid is an important one. This observation appears to be new. Cilia have been known to exist on the ependymal epithelium since the time of Purkinje. Yet both anatomists and physiologists seem not to have suspected that these cilia have any relation to the motion of the cerebrospinal fluid, as shown by the latest reviews of the literature on the structure of ependyma (Wislocki, 1928; Agduhr, 1932) and on the circulation of the cerebrospinal fluid (Abderhalden, 1927; Plaut, 1927).

According to Studnicka (1900), cilia are present on the ependyma with great regularity in lower vertebrates and during fetal life in mammalia. But, in adult mammals, they are present only in restricted areas. These facts suggest that ciliary movement may not play any part in the circulation of the cerebrospinal fluid in mammals. However, records thus far published by different investigators of brain pulses taken from trephine holes on the skull of different mammals including man throw some doubts on this suggestion. All records register many highly irregular oscillations, in addition to the waves corresponding either to the heart beat or to the respiratory rhythm. Such irregular oscillations have not yet been explained, and most probably are caused by the synchronized movements of a great many ependymal cilia. Furthermore, observations on rats also are against the above-mentioned suggestion. In our experiments we added a few drops of diluted frog's blood to the mammalian saline solution in the exposed third or fourth ventricle of a removed brain, and found the frog's red blood corpuscles moving in the same manner as those introduced into the ventricle of an anuran. The whirling about of the frog's red blood cells in the rat's ventricle certainly indicates that the cilia on the ependyma are in constant motion, although we have not succeeded in observing directly the movements of the cilia on a piece of the roof of the fourth ventricle removed from the rat. Thus, ciliary movement most probably plays the same rôle in the circulation of the cerebrospinal fluid in mammalia just as in amphibia.

#### SUMMARY

Observations are made on the movements of some dark particles in the brain ventricles of living anuran larvae. The dark particles have their origin in the pigmented cells of the ependymal covering over the inner wall of neural tube. They persist throughout the larval life; and they are absent in the adults. The movements of dark particles are due to the local currents in the cerebrospinal fluid generated by the motion of the cilia on epithelial cells lining the ventricles.

Observations on frogs and toads show that movements of the cilia on the ependyma also produce local currents in the cerebrospinal fluid.

The significance of ciliary movements as a hitherto neglected factor in the circulation of the cerebrospinal fluid within the brain ventricles is discussed.

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# URINE DILUTION AND CONCENTRATION TESTS IN ADRENALECTOMIZED DOGS

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Evidence has accumulated which indicates that during advanced adrenal insufficiency the ability of the kidney to perform its normal functions is diminished. There is a reduction of the elimination of urea, so that this substance accumulates in the blood (1). Likewise, the renal excretion of potassium is reduced and its quantity in the blood is increased (2, 3). At the same time the power of the kidney to retain sodium and chloride is diminished, so that these substances are lost from the body (3, 4, 5). The present investigation was undertaken with a twofold aim: first, to determine the power of the kidney to do its work in a general rather than a specific manner in the absence of adrenal cortical hormone, but with the animal in good condition; second, under similar circumstances to determine the ability of the kidney to excrete excess chloride.

**PROCEDURE AND METHODS.** To achieve the purposes of this research, urine dilution and concentration tests and a renal chloride concentration test were carried out on dogs before and after adrenalectomy. After adrenalectomy these tests were performed when the animals were maintained in good condition as described subsequently without cortical hormone. Healthy dogs weighing from 12 to 15 kgm. were used. The adrenal glands were removed with the usual surgical technic under ether anesthesia at separate operations. An interval of three weeks or more was allowed between operations. As a routine all animals were dewormed with tetrachlorethylene prior to removal of the second adrenal.

It was important in this study to maintain the animals on the same diet during the kidney function tests both before and after adrenalectomy so that alterations in this factor would not complicate the tests. A high sodium-low potassium diet was used. It was composed of casein, 12 per cent; whole wheat flour, 15 per cent; sucrose, 10 per cent; lard, 10 per cent; butter, 5 per cent; yeast, 2 per cent; cod liver oil, 1 per cent; sodium chloride, 2.5 per cent; sodium citrate 1.5 per cent; bone ash, 1 per cent; and water, 40 per cent. The ingredients were thoroughly mixed and then baked in a slow oven to the consistency of soft cake. The animals were fed 300 to 350 grams of this diet per day and on this quantity they maintained their body weight or gained. At times, particularly immediately after adrenalectomy or when early signs of insufficiency were present, it was necessary to feed the diet by hand. As a routine, tablets of yeast concentrate

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were added to the cooked diet rather than cooked with the diet. In order to increase the sodium intake further, sodium chloride was added to the drinking water in quantities sufficient to make a 0.5 per cent solution. The total sodium intake was about 6.0 grams per day while the potassium intake was less than 300 mgm. per day.

The intact animals were placed on the high sodium-low potassium diet for at least three days before a series of control tests was run. The urine dilution and concentration tests and the chloride concentration test were performed in series with one day rest between the dilution and concentration tests. The urine chloride concentration test was carried out immediately after either the dilution or concentration test, before any food or water had been given to the dog. Tests were performed on some animals after one of the adrenal glands had been removed. No difference was found between these and tests on intact animals.

After the control tests had been completed satisfactorily, the second adrenal was removed. After the operation the high sodium diet was supplemented by the intravenous administration of hypertonic solutions of sodium chloride and glucose and by the subcutaneous injection of cortical extract or desoxycorticosterone acetate in sesame oil. Injections of hypertonic solutions of salt and glucose were discontinued after two or three days. Treatment with cortical extract or desoxycorticosterone acetate was stopped eight to ten days after operation when the wound was well healed and the animal in good condition. A period of seven to ten days was allowed to elapse before any renal function tests were performed to insure that all of the cortical substitute had been destroyed or excreted.

*Urine dilution test.* After a twenty-four hour fast, the animals were catheterized and then given 300 cc. of water by stomach tube. Catheterization was repeated at hourly intervals for four to seven hours. The volume, specific gravity, and chloride concentration of the urine were determined for each hour sample.

*Urine concentration test.* The concentration test was started in the morning by catheterization of the animal and removal of water from the cage. The standard daily ration was then given and from this time until the end of the test the animal did not receive any further food or water. As a routine animals were catheterized at least morning and evening. The volume, specific gravity, and chloride concentration were determined for each sample of urine.

*Urine chloride concentration test.* In an attempt to determine the maximal ability of the kidney to concentrate chloride in the urine a test was developed in which a hypertonic solution of sodium chloride was given by vein and the chloride concentration in the urine was followed at definite intervals. This test was carried out immediately after either the urine concentration or urine dilution test. The procedure followed was to inject 350 cc. of 4.5 per cent solution of sodium chloride into the saphenous vein at a constant rate of 10 cc. per minute. Blood samples were taken prior to injection, just before the end of the injection, and thirty minutes after the injection was completed. A catheter was introduced into the bladder and left in place for the duration of the test. The bladder was drained before the injection was started and at ten minute intervals until the

diuresis became less than about 1 cc. per minute when the collection time was lengthened to twenty to thirty minutes to allow larger samples for analysis. As a rule the ten minute samples were obtained for about two hours and sampling at the longer interval continued for a further hour.

The specific gravity of the urine was determined by weighing samples delivered from a 1 cc. syringe-pipet of the Krogh-Keys type. To offset the loss of weight by evaporation during weighing the urine was delivered into a 25 cc. Erlenmeyer flask counterbalanced by a similar flask containing water. All samples were weighed to 0.1 mgm. on an analytical balance. Single and triple deliveries from the syringe-pipet were used to determine the weight of the standard urine volume. Experiments showed that the reproducibility of results of triple deliveries was slightly more accurate than for single deliveries; therefore whenever the urine samples were sufficiently large, triple deliveries were used. The standard deviation determined by triple delivery was 0.5 mgm. Any figures considered significant in this work were many times as large as this figure. Corrections for changes in weight due to variations in the temperature at the time of weighing were made by use of standard tables of water density.

Throughout this study all animals were kept in cages suitable for urine collection. During the various tests all samples of urine were obtained by catheterization. To insure complete emptying of the bladder, the urine was withdrawn through the catheter under gentle suction with a 50 cc. syringe and light pressure was applied to the lower part of the abdomen. Blood samples were taken from the saphenous or jugular vein into oiled syringes containing heparin as an anticoagulant. The amount of urea in the blood was used as one indication of the condition of the adrenalectomized animals. It was estimated by the urease aeration method of Van Slyke and Cullen (6). Urine chloride estimations were done by the Van Slyke modification of the Volhard-Harvey titration method. The Van Slyke and Sendroy application of the open Carius method was used to determine the plasma chloride. Blood cell volume ratios were estimated by centrifuging 10 cc. samples of blood in recalibrated graduated centrifuge tubes at 2800 r.p.m. for thirty minutes.

All of the animals used in this study eventually died with the typical signs of adrenal insufficiency and at necropsy no adrenal tissue was found.

RESULTS. I. *Dilution tests.* Seventeen dilution tests were carried out on eight healthy intact dogs maintained on the high sodium-low potassium diet for at least three days before performance of the test. The results of these control tests were compared with those obtained in fourteen tests on five adrenalectomized animals maintained on the same regimen. Before the water for the test was given, the bladder was emptied. The specific gravity of this initial urine was variable, ranging from 1.039 to 1.007 in the intact animals and from 1.034 to 1.011 in the adrenalectomized. Despite this wide variation of the initial specific gravity values, the low levels reached during the first and second hour after administration of the water were quite constant in both the normal and the adrenalectomized animals (table 1). During the third hour the specific gravity rose sharply in both the normal and adrenalectomized dogs.

When the values of the specific gravity of the urine are charted against time, a

curve for the dilution test is obtained. In each animal the mean curve of all the tests performed before adrenalectomy was compared with the mean curve of all the tests performed after adrenalectomy. In each animal the mean curve fell to a lower level before adrenalectomy than after adrenalectomy.

Comparison of the various *mean* specific gravity values in the normal and adrenalectomized animals suggested some reduction of the ability of the adrenal-

TABLE 1

*Specific gravity of urine during dilution tests in normal and adrenalectomized dogs*

PERIOD	NUMBER		SPECIFIC GRAVITY OF URINE			
	Tests	Dogs	Maximum	Minimum	Mean	Standard deviation
Normal animals						
1st hour	17	8	1.0110	1.0005	1.0042	0.0032
2nd hour	17	8	1.0063	1.0002	1.0033	0.0018
3rd hour	13	7	1.0134	1.0010	1.0071	0.0046
4th hour	11	6	1.0209	1.0050	1.0136	0.0046
Adrenalectomized animals						
1st hour	14	5	1.0303	1.0057	1.0142	0.0072
2nd hour	13	5	1.0211	1.0017	1.0084	0.0064
3rd hour	14	5	1.0185	1.0037	1.0096	0.0047
4th hour	14	5	1.0215	1.0043	1.0110	0.0052

TABLE 2

*Urine dilution tests during adrenal insufficiency*

DOG	URINE SAMPLE		CLINICAL CONDITION
	Time taken during test	Specific gravity	
	<i>hours</i>		
H-1	2	1.022	Anorexia, vomiting, progressive weakness
	2	1.011	Anorexia, vomiting, listlessness
F	2	1.015	Anorexia, plasma K 31.3 mgm. per 100 cc.
	3	1.013	24 mgm. urea nitrogen per 100 cc. blood
S	2	1.021	Anorexia, vomiting, progressive weakness
	3	1.019	

ectomized dog to produce a dilute urine. Further study showed that such a conclusion was unjustified. The results of the tests on the adrenalectomized animals were more variable than those obtained from the intact animals. It was found that the ability of the adrenalectomized animals to dilute the urine was dependent on the success with which they were maintained on the high sodium-low potassium diet. When maintenance was inadequate, the adrenalectomized animals were unable to dilute the urine below a specific gravity of 1.010 (table 2).

On the other hand, when the adrenalectomized animal was in the best clinical condition the results of the dilution test were practically the same as those obtained in the intact animal.

To emphasize this finding further, the best performance of each dog before adrenalectomy was compared with the most satisfactory performance obtained after adrenalectomy (fig. 1). In each animal the greatest dilution of the urine occurred before adrenalectomy but the differences in the lowest specific gravity values obtained before and after adrenalectomy (0.0002, 0.0004, 0.0009, 0.0018 and 0.0050 respectively) were, with the exception of the last instance, so slight that no special significance has been attached to them. Three of the differences are within the range of error of the specific gravity estimation. The experiments indicate that the ability of the kidney of the adrenalectomized dog to dilute the urine is not impaired when the animal is maintained in good clinical condition on a high sodium-low potassium diet without cortical hormone. When signs of

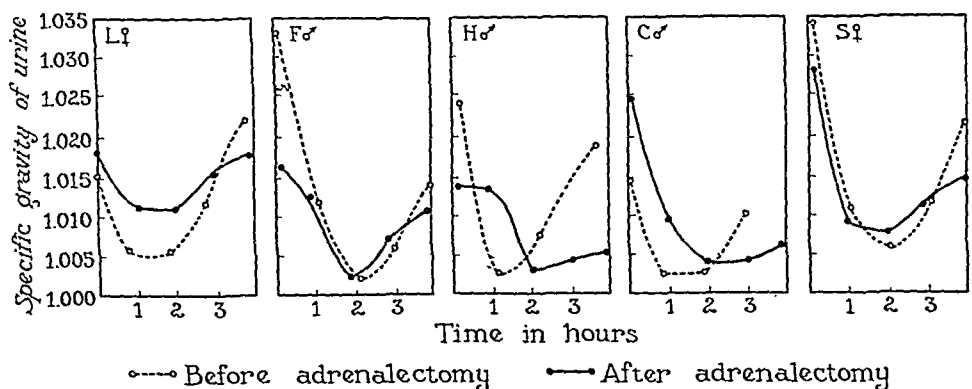


Fig. 1. Specific gravity of urine during urine dilution tests, before and after adrenalectomy in dogs L, F, H, C and S maintained on a high sodium-low potassium diet without cortical hormone.

adrenal insufficiency supervene there is a rapid deterioration in the urine diluting power of the kidney.

II. *Concentration tests.* Early in the study it became evident that the degree of concentration reached in the urine varied with the environmental conditions. As might be expected, during hot summer weather the concentration of the urine was considerably greater than during the remainder of the year when moderate temperatures prevailed in the animal quarters. The concentration tests therefore have been separated into two groups, the moderate temperature tests and the hot weather tests.

During the moderate temperature tests the specific gravity of the urine of the intact animals maintained on the high sodium-low potassium diet usually exceeded 1.035 forty hours after food and water had been taken from the cages (fig. 2). Fifteen tests of more than twenty hours' duration were carried out on the intact animals. In twelve of these a specific gravity of more than 1.035 was observed at some time during the test. In the remaining three a specific gravity

of more than 1.030 was obtained. With the intact animal, a concentration of more than 1.032 was attained in every test which was continued for more than thirty hours. Only two tests at moderate temperatures were carried out on adrenalectomized animals. In these, the specific gravity of the urine did not exceed 1.028 during the first seventy hours of the test and the highest concentration reached after seventy hours was 1.032. The results suggested some reduction in the power of the kidney of the adrenalectomized dog to secrete a concentrated urine. Definite differences, however, were encountered in the hot weather tests and for this reason the tests at moderate temperatures were discontinued.

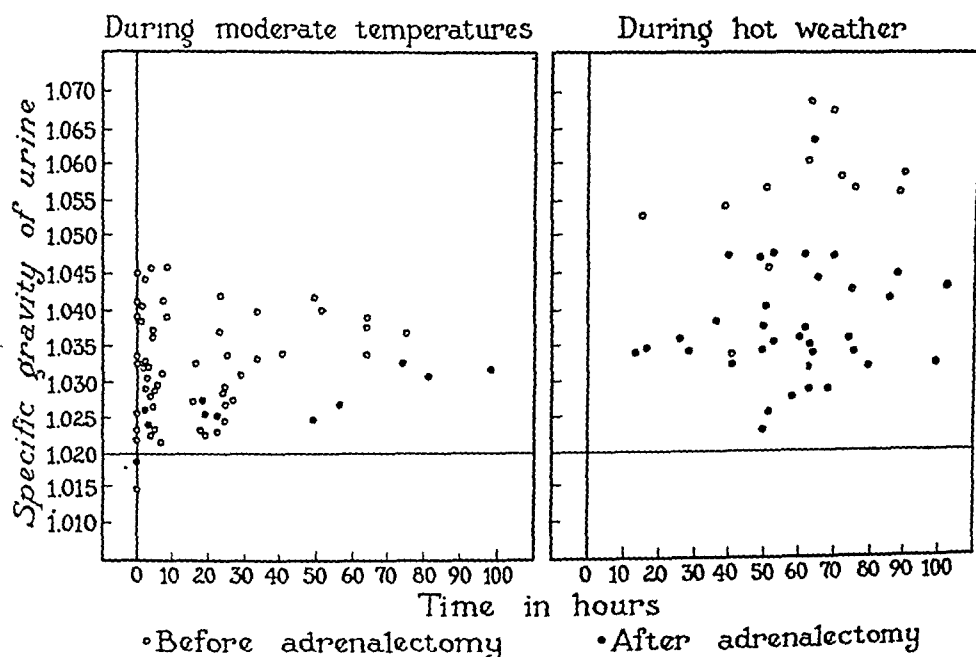


Fig. 2. Specific gravity of the urine of intact and adrenalectomized dogs during urine concentration tests in hot and moderate weather. All animals maintained on high sodium-low potassium diet without cortical hormone.

The concentration tests during high environmental temperatures were controlled by studies on three intact dogs. All achieved urine concentrations of more than specific gravity 1.058 (fig. 2). The highest specific gravity was 1.066 and the average of the series was 1.062. In contrast, the highest urine specific gravity reached in seven tests on five adrenalectomized animals during the same hot weather period was 1.046 (fig. 2).

One of the adrenalectomized female dogs had been maintained in excellent condition on the high sodium-low potassium diet alone for more than thirty days before tests were run. This animal then had the appearance and vigor of a normal intact dog. Its concentration tests were carried out during the hottest summer weather, when the temperature reached 100°F. or more for several consecutive days. The maximal specific gravity values reached during these

tests were 1.041 to 1.046. These values are 0.010 to 0.015 less than the maximal concentration obtained in intact dogs.

During the course of these tests it was noticed that the ability of the adrenalectomized animals to elaborate a concentrated urine could be correlated with the general condition of the animal. All the results reported in the previous paragraphs were obtained on animals in good clinical condition. When signs of insufficiency were present some reduction of the concentrating power was evident.

The results of the concentration tests indicate that the power of adrenalectomized dogs maintained on a high sodium-low potassium diet without cortical hormone to concentrate urine is reduced even when these animals are in excellent general condition.

III. *Chloride concentration tests.* There is adequate evidence of impairment of the ability of the kidney of adrenalectomized animals to produce a urine of low chloride content, but little is known of the upper limit of chloride concentrating power in these animals. The chloride concentration test was devised to determine this maximal chloride concentrating power in adrenalectomized animals maintained in good health, without cortical hormone, on the high sodium-low potassium diet.

In performance of the test, after the bladder had been emptied a 4.5 per cent solution of sodium chloride was injected intravenously at the rate of 10 cc. per minute for thirty-five minutes. In the preliminary tests on intact dogs the hypertonic solution was injected under two contrasting states of body hydration. This was accomplished by performing the test immediately after either the urine dilution test or the urine concentration test. When the chloride concentration test followed the dilution test, excess water had been administered only a few hours previously but when the chloride concentration test followed the urine concentration test, no water had been taken for at least fifty hours. It was soon found, as might be anticipated, that greater concentrations of chloride in the urine were obtained when the hypertonic saline solution was injected immediately after a concentration test than when the injection followed a dilution test. As a routine, therefore, the hypertonic salt solution was given after a concentration test.

*Urine volume production.* About twenty minutes after the injection had been started, the rate of urine production increased sharply. The maximal rate usually was reached during the first hour, after which urine production gradually declined (fig. 3). In the intact animal when the salt was given after a dilution test, the diuresis was more prolonged and the maximal rate of production of urine often higher than when the injection followed a concentration test (fig. 3). The total volume of urine formed was also greater when the salt was given immediately after a dilution test than when it was given after a concentration test.

When the salt injection followed a concentration test, the maximal rate of production of urine ranged from 4.6 to 8.7 cc. per minute in the intact animals and from 2.7 to 5.5 cc. per minute in the adrenalectomized animals. The decline of the diuresis was somewhat more rapid in the intact than in the adrenalecto-

mized dogs, so that by the end of the test there was not much difference between the total volumes of urine eliminated by the intact and adrenalectomized animals (fig. 3a).

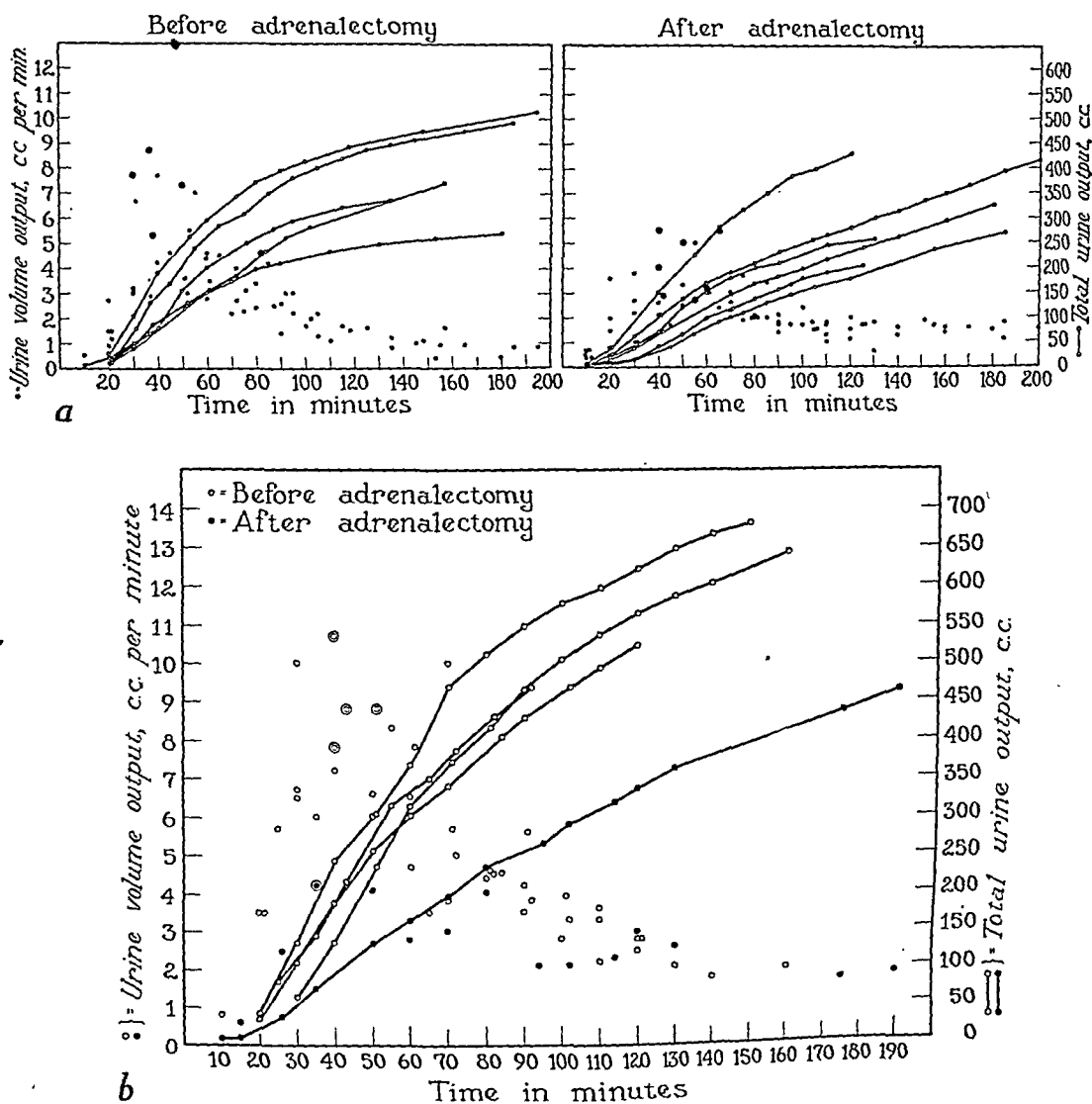


Fig. 3. Rate of production of urine and total volume of urine produced during chloride concentration tests in intact and adrenalectomized dogs maintained on high sodium-low potassium diet without cortical hormone. *a*, When chloride concentration tests were done immediately after urine concentration tests; *b*, when chloride concentration tests were done immediately after urine dilution tests. Double circles indicate the maximal rate of urine production in individual tests.

In the adrenalectomized dogs only one of the chloride concentration tests was done immediately after a dilution test. The rate of production of urine and the total volume of urine produced during this single test were distinctly less than those observed during the four control tests carried out under similar circumstances (fig. 3b).

In general, there was a definite tendency for the rate of production of urine in the adrenalectomized animals to rise slowly to a maximum and remain fixed at this level throughout the test. As a rule this rather fixed rate of production of urine in the adrenalectomized dogs was less than the maximal rate of production of urine observed in intact animals.

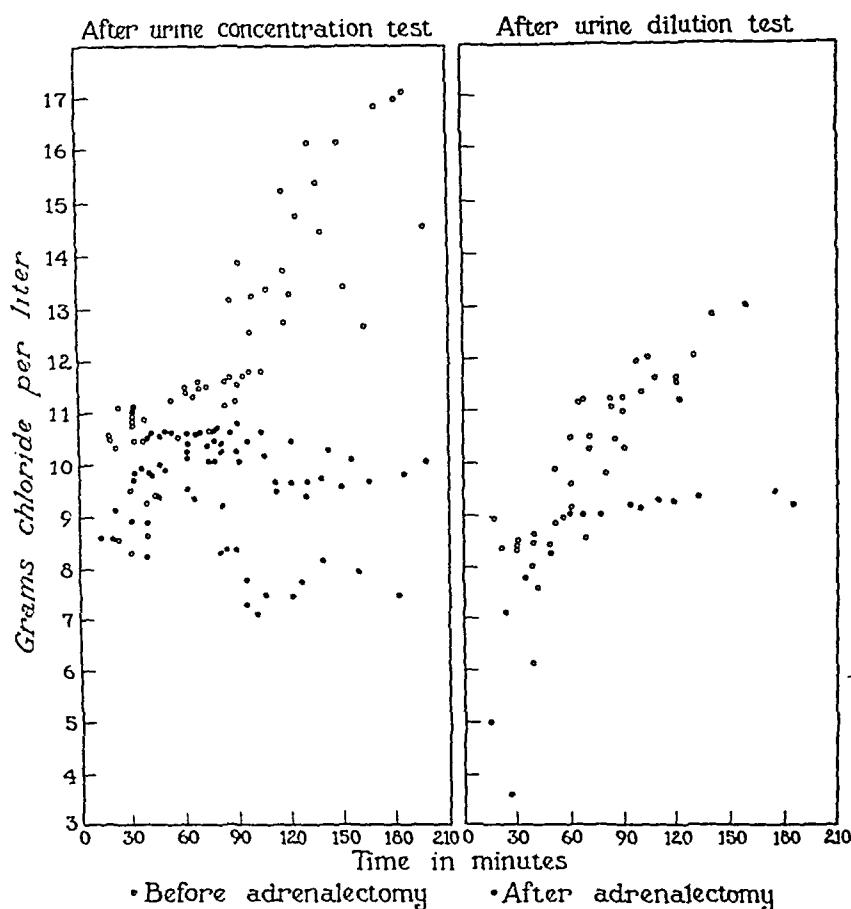


Fig. 4. Chloride concentrations in the urine of intact and adrenalectomized dogs during chloride concentration tests which were done immediately after urine concentration tests or urine dilution tests. Animals maintained on high sodium-low potassium diet without cortical hormone.

*Urine chloride concentration.* During the period of diuresis, the chloride content of the urine varied over approximately the same range of 6 to 10 grams per liter in both the intact and adrenalectomized dogs (fig. 4). During the later minutes of the test, after the diuresis had subsided, there was a marked difference between the chloride concentrations attained by the intact animals and those reached by the adrenalectomized animals. In the intact dogs after the period of diuresis, the chloride concentration rose to values varying from 13.2 to 17.9 grams of chloride per liter when the salt was given after a concentration test and from 10.7 to 13.0 grams when the salt was given after a dilution test. In



contrast to the intact animals the urine chloride concentration of the adrenalectomized animals did not rise after cessation of the diuresis but remained relatively fixed at levels far below those observed in the intact animals (fig. 4). Throughout the tests the highest urine chloride concentration found in an adrenalectomized animal was 11.6 grams per liter. In all other instances the maximal chloride concentration attained by an adrenalectomized animal was 11.0 grams per liter or less. The difference between the intact and adrenalectomized animal was most noticeable when the tests carried out before and after adrenalectomy were compared in the same dog (fig. 5). The experiments indi-

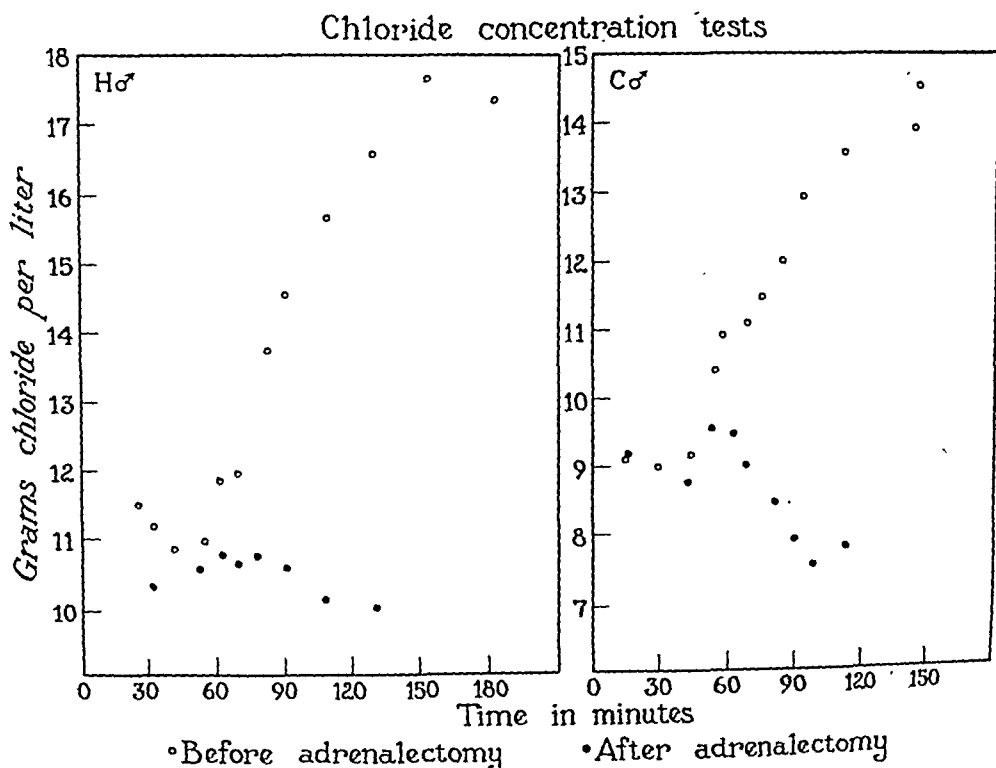


Fig. 5. Concentrations of chloride in the urine of two dogs, H and C, during chloride concentration tests before and after adrenalectomy. Animals maintained on high sodium-low potassium diet without cortical hormone.

cated that in the adrenalectomized animal, even when well maintained on a high sodium-low potassium diet without cortical hormone, the kidney had lost its ability to concentrate chloride in the urine above a ceiling of approximately 11 grams of chloride per liter of urine.

*Urine chloride output.* The changes in the chloride output per minute followed roughly the same pattern as the changes in rate of production of urine. In all instances, the most rapid elimination of chloride occurred during the period of diuresis, after which it gradually declined as production of urine was reduced (fig. 6). In the intact animals the rapid rate of elimination of chloride was continued for a longer period when the chloride was given after a dilution test than when administered after a concentration test. As a result, in the intact

animals the total chloride eliminated during the test was generally greater when the salt was injected after a dilution test (fig. 6). In the adrenalectomized dogs the rate of chloride elimination was generally slower during the first two hours

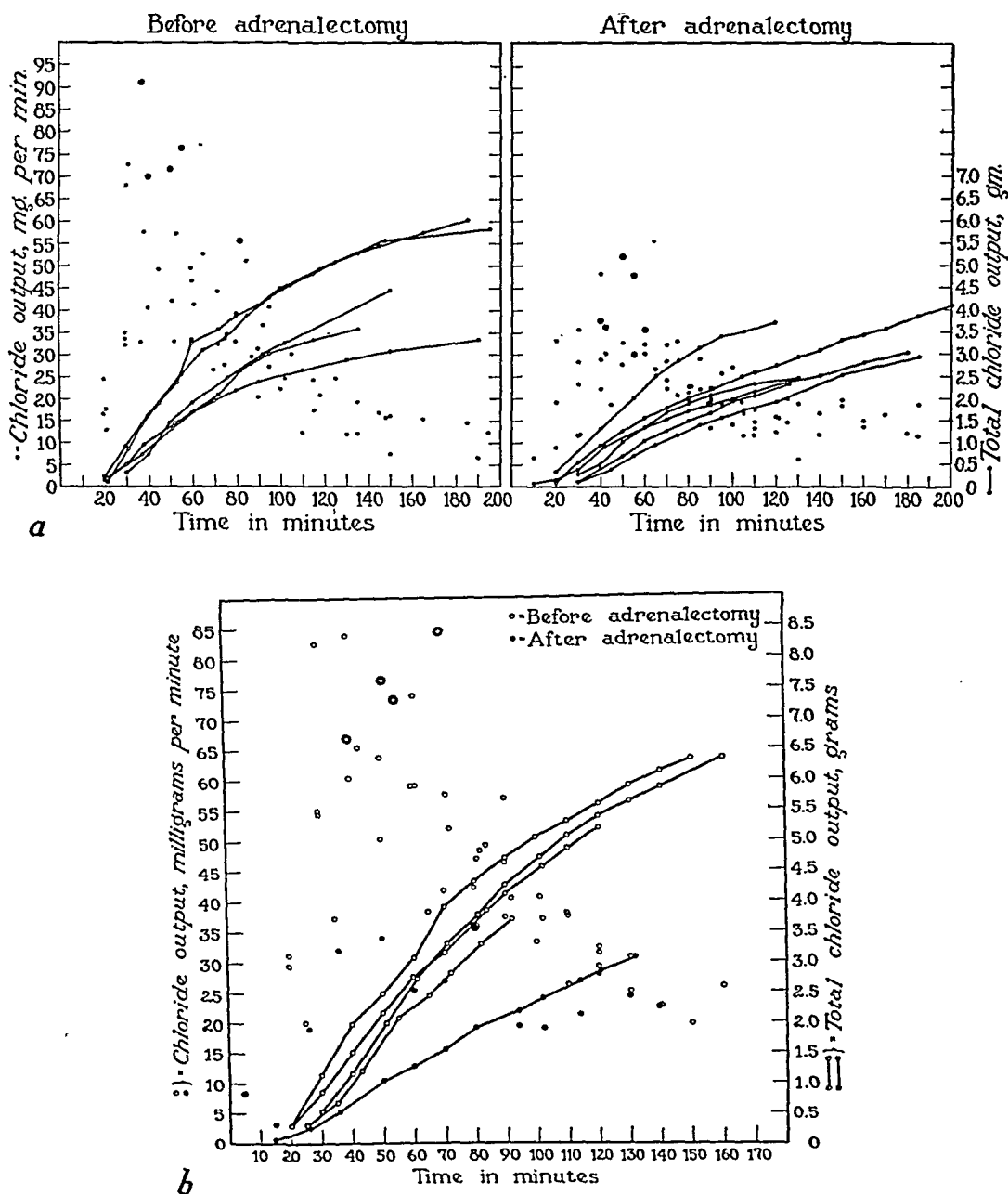


Fig. 6. Rate of chloride output and total chloride output in the urine during chloride concentration tests in dogs before and after adrenalectomy; *a*, when chloride concentration test was done immediately after a urine concentration test, and *b*, when chloride concentration test was done immediately after a urine dilution test. All animals maintained on high sodium-low potassium diet without cortical hormone. Double circles indicate maximal rate of chloride output in each test.

of the test, than in the intact animals. The maximal chloride output per minute was always less in the adrenalectomized than in the intact animals. Throughout all the tests the rate of elimination of chloride in the adrenalectomized dogs was more or less fixed in comparison with that of the intact animals (fig. 6). The total quantity of chloride eliminated during the tests was somewhat greater in the intact than in the adrenalectomized animals although the differences were not striking. The greatest amount of chloride was excreted when the salt injection was preceded by the administration of water (fig. 6b). The total quantity of chloride eliminated during the two to three hours of the test was always less than the quantity injected.

It seemed possible that the differences between the normal and the adrenalectomized animal might be due to differences in the concentration of chloride in the plasma delivered to the kidney. Blood samples were drawn thirty and sixty minutes after the beginning of the injection and the cell volume and plasma

TABLE 3

*Red cell and plasma chloride concentrations during the chloride concentration test*

DOG	PER CENT RED CELLS			MG. CHLORIDE PER 100 CC. PLASMA		
	Before test	Minutes during test		Before test	Minutes during test	
		30	60		30	60
Before adrenalectomy						
H-2	54.6	38.4	42.8	392	503	441
S	46.0	31.8	37.8	419	481	470
After adrenalectomy						
L	52.0	42.3	42.1	391	485	473
H-2	57.7	34.8	41.0	390	495	456
L	46.8	42.9	39.0	391	475	483
L	49.0	32.5	39.0	396	465	398

chloride concentration determined. No marked differences were noticed between the values obtained for the intact and the adrenalectomized animals (table 3).

The data indicated that the kidney of the adrenalectomized animal had lost some of its flexibility despite the administration of adequate quantities of sodium chloride. In these experiments the maximal urine chloride concentrating power of the adrenalectomized dog, maintained in good health on the high sodium-low potassium diet without cortical hormone, was less than that of the intact dog.

COMMENT. In this study an attempt has been made to determine the extent to which the kidney, deprived of adrenal cortical hormone but supplied with sodium chloride, can change the concentration of solids in the urine. The specific gravity of the urine was used because it gives some measure of the ratio of water to dissolved substances. When subjected to a dilution test the kidney of the adrenalectomized animal was found to be able to elaborate as dilute, or

nearly as dilute, a urine as the intact animal. In the presence of sufficient sodium chloride, lack of cortical hormone did not limit seriously the ability of the tubule to reabsorb the crystalloids and leave the water of the glomerular filtrate.

In the concentration test some measure was obtained of the ability of the tubule to reabsorb water selectively and thus to allow accumulation of dissolved substances in the urine. During the hot weather tests the tubule without cortical hormone was unable to carry out this differential reabsorption of water sufficiently to elevate the specific gravity to the values obtained in the normal dogs. It seems unlikely that there was sufficient difference in the pattern of solids excreted in the urine of the intact and adrenalectomized dogs to account for the differences in the specific gravities of the urine samples. Apparently the high sodium-low potassium diet did not restore tubular function completely. It should be pointed out, however, that in this test the difference between the performance of the intact and adrenalectomized animals was not great. On the basis of general clinical experience with the urine concentration test the adrenalectomized animals on the high sodium-low potassium diet showed a degree of urine concentrating power usually considered sufficient for ordinary body needs. Despite this rather satisfactory over-all performance by the kidney the experiments definitely point to a reduction in the power of the tubule to reabsorb water selectively.

This was emphasized again in the chloride concentration tests in which the tubule without cortical hormone was unable to reabsorb water differentially sufficiently to raise the concentration of chloride in the urine to more than approximately 11 grams per liter. It may be considered that in these latter experiments the tubule was unable to refuse selectively to reabsorb chloride rather than unable to reabsorb sufficient water selectively. No matter which view is taken, the data indicate some reduction of the tubular function of the kidney supplied with ample sodium chloride but deprived of cortical hormone.

During the dilution tests the rate of formation of urine was, as a rule, somewhat slower in the adrenalectomized animals than in the intact animals. The water of the test was given by mouth and the slower rate of elimination in the adrenalectomized animals was possibly partly due to a reduced rate of absorption of the water from the gastro-intestinal tract (7). In the light of the findings of Harrison and Darrow (3) the possibility of a maximal rate of glomerular filtration somewhat less than that of the normal animal may also be considered as a possible cause of the slower rate of elimination in the adrenalectomized animals. A smaller volume of glomerular filtrate with a resulting slower passage through the tubules may have assisted the kidney of the adrenalectomized animals to produce an extremely dilute urine. No significant difference was noticed in the concentration of chloride in the urine during the dilution tests of the intact and adrenalectomized animals. Both groups of animals had been receiving large quantities of chloride in the high sodium-low potassium diet up to the time of giving the test. It seems probable that excess chloride was present in the body and that for this reason no difference in chloride conservation by the kidney was noticed during the test.

Willson and Sunderman (8) observed only a moderate increase of chloride

and sodium in the urine of a patient suffering from Addison's disease during a period of water deprivation. This observation suggested a diminution of the ability of their patient to concentrate sodium and chloride in urine. The present study has shown quite definitely that the maximal urine chloride concentrating power of adrenalectomized dogs, maintained in good health on the high sodium-low potassium diet without cortical hormone, is less than that of the intact dog.

Maintenance of mineral balance on an average diet necessitates the elimination of potassium and at times the conservation of both sodium and chloride. To accomplish this the kidney is required to concentrate potassium and if necessary dilute sodium and chloride in the urine. Retention of potassium and loss of sodium and chloride in the animal suffering from adrenal insufficiency have been explained as being due to a specific inability of the kidney to meet these requirements by failing to produce the necessary concentration gradients between plasma and urine (3). In this study it has been shown that the renal dysfunction of adrenalectomized animals is not limited to failure to concentrate potassium and to dilute sodium and chloride in the urine but also includes a reduction in ability to concentrate chloride in the urine. For it was found during the chloride concentration tests that the adrenalectomized animal failed to concentrate chloride to the extent of the normal and as a result there was actually an abnormal retention of this ion. Thus when high concentrations of chloride are required in the urine, the kidney of the adrenalectomized animal behaves toward chloride as it has been shown to behave toward potassium when high concentrations of potassium are required in the urine.

#### SUMMARY AND CONCLUSIONS

The kidney function of adrenalectomized dogs maintained in good condition on a high sodium-low potassium diet without cortical hormone was tested by a urine dilution test, a urine concentration test and a chloride concentration test and the results were compared with those obtained in normal animals maintained on the same diet.

1. With the urine dilution test, when the adrenalectomized animals were well sustained without cortical hormone on the high sodium-low potassium regimen, as dilute or nearly as dilute a urine was obtained as from the intact animals.

2. During hot weather the adrenalectomized animals, deprived of cortical hormone but well maintained on the high sodium-low potassium diet, were unable to produce as concentrated urine as intact animals.

3. In the case of the chloride concentration tests, the maximal chloride concentration was limited in the adrenalectomized animals to levels less than 11.6 grams per liter of urine, while in the normal animal the concentration of chloride in the urine rose to values considerably greater than this level. In these experiments the maximal chloride concentrating ability of the kidney in adrenalectomized animals maintained without cortical hormone on the high sodium-low potassium diet was less than that of the intact animal.

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# ABSORPTION AND DISPOSITION OF GLUCOSE IN THE CHICK

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While extensive studies have been reported on the absorption and disposition of carbohydrates from the gastro-intestinal tract of mammals, very few quantitative studies have been made in birds.

Henry et al. (1) determined the absorption coefficient for glucose in adult fowls. These authors did not describe their technique in detail but presumably followed the Cori (2) procedure. Glucose solutions varying in concentration from 13.5 per cent to 54 per cent were used for an absorption period of 20 minutes. The absorption coefficient found for the 3 solutions were, respectively, 209, 204 and 140 mgm. per 100 grams per hour. They found the absorption rate to vary with the concentration of glucose administered, but it should be pointed out that values obtained for such short absorption periods are likely to be unreliable.

Emslie and Henry (3), also employing the Cori procedures, studied the absorption of glucose, galactose and lactose in young chicks. The sugars were administered in 56 per cent aqueous solution and absorption periods of 1.5, 3.0, 4.5 and 6.0 hours were allowed. These workers found the three sugars to be absorbed at the following relative rates: glucose > galactose > lactose. There was a progressive decrease in the rate of absorption of each sugar with time, that of glucose decreasing from 655 mgm. at 1.5 hr. to 245 at 6.0 hrs.

A comparison of the relative velocities of the absorption of different sugars in the rat and pigeon is contained in a brief note by Westerbrink (4). This author has carried out experiments on rats and pigeons which for 5 days had been on a diet consisting only of fat, casein and salts (no vitamins). The rats showed the following series of relative absorption rates: d-galactose: d-glucose: d-fructose: d-mannose: l-xylose: l-arabinose: 108: 100: 42: 15: 13: 2; the pigeons 115: 100: 55: 33: 33: 16. The results with the rat were similar to those of Cori and there was observed no difference in relative absorption rates between the rat and the pigeon.

Recently Morando (5) has reported that ligation of the pancreatic ducts or pancreatectomy in ducks and pigeons was followed by a reduction of intestinal glucose absorption.

In the present investigation the rate of absorption of glucose was studied in young White Leghorn chicks varying in weight from 150 to 250 grams. In addition some information was also obtained on the disposition of the absorbed glucose by observing the changes in the levels of blood glucose, muscle and liver glycogen.

<sup>1</sup> The data forming the basis of this paper were taken from a dissertation submitted by W. R. C. Golden in partial fulfillment of the requirement for the degree of Doctor of Philosophy, Yale University, 1940.

PROCEDURES. The selection and care of the experimental chicks as well as the chemical methods employed are described elsewhere (6).

The general procedure employed for the study of the absorption rate was essentially that outlined by Cori (2) for the study of absorption in the rat.

Merck's reagent grade d-glucose, dissolved in distilled water was used and the chicks were fasted 24 to 30 hours before the administration of glucose. A no. 8 (French scale) rubber catheter attached by means of a shortened 15-gauge hypodermic needle to a syringe of 5 to 10 cc. capacity was used to administer the glucose.

After filling with the glucose solution the catheter was gently inserted into the esophagus and pushed downward until the end was palpable in the crop. The full capacity of the syringe was then delivered into the crop of the chick and the catheter immediately withdrawn. In some instances the end of the catheter went past the crop into the proventriculus. In such cases, the glucose solution must have been delivered into the proventriculus and gizzard but, since in the fasted chick the crop discharges material almost immediately into the gizzard (1), the glucose solution must have arrived at the absorbing portion of the gut in either case at approximately the same time.

To measure the quantity of glucose given, a similar charge from the syringe was delivered into a volumetric flask, diluted to volume and aliquots were taken for analysis.

The time of delivery of the glucose solution into the crop of the chick was recorded and 5 minutes before the expiration of the absorption period the chick was anesthetized, with nembutal given intraperitoneally. When anesthesia was complete, the upper end of the esophagus and lower end of the rectum were ligatured to prevent escape of fluid during manipulation. Then, in successive order, a sample of pectoral muscle and a portion of liver were taken in the usual manner for glycogen determinations and following this, blood was obtained.

As soon as the tissues and blood were obtained, the esophagus was severed anteriorly and the rectum posteriorly to the respective ligatures. The entire gastrointestinal tract was then drawn out, stripped of adhering mesenteric tissues, and transferred to a dish containing about 100 cc. of hot water to which had been added a liberal quantity of powdered sodium fluoride. The entire tract was slit lengthwise and then successively extracted with 4 or 5 portions of hot water, which were transferred to a 500 cc. volumetric flask. Twenty-five cubic centimeters of 10 per cent  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  and 25 cc. of 0.5 N NaOH were next added in respective order with thorough mixing after each addition. The mixture was cooled, made up to volume with water, and after thorough mixing was filtered and a small portion of the filtrate was collected for glucose determinations. Finally, the residual portion of the liver not used for glycogen analysis was taken from the chick and weighed so that the total weight of the liver would be known.

The rate of absorption of the glucose was expressed as the "absorption coefficient" according to Cori's definition: that is, the number of milligrams of glucose absorbed per hour per 100 grams body weight (fasted).



EXPERIMENTAL RESULTS AND DISCUSSION. Before studying the rate of absorption of glucose from the intestinal tract of the fasted chick according to the procedure described above, two questions relating to the method employed had first to be answered. They were:

1. Does the gastro-intestinal tract of a 24-hour fasted chick contain significant quantities of reducing material?
2. Can administered glucose be recovered quantitatively if no time is allowed for absorption to take place?

The first question was answered by removing the intestinal tracts of several fasted chicks, extracting them, precipitating the proteins as described and analyzing the filtrates so obtained. The results indicate that the reducing material of the intestinal tract recoverable by this procedure is negligible. The actual quantities, expressed as glucose, found in 6 chicks were 20, 30, 23, 13, 15 and 10 mgm. No correction for this amount was applied in the calculations.

The answer to the second question was sought in the following manner:

A known amount of glucose was discharged into the crop of each of several heavily anesthetized chicks on the presumption that absorption would be very slow under such conditions. The intestinal tracts of the birds so treated were removed immediately following the administration of the glucose and extracted and analyzed in the usual manner. The glucose recovery values for 3 chicks were 95, 96 and 98 per cent. Emslie and Henry (3) have performed a similar experiment, with the exception that their birds were not anesthetized. They obtained recovery values ranging from 86 to 95 per cent. Some of the lower recoveries may be explained, as these authors suggested, by the fact that in the non-anesthetized birds absorption of the glucose may have already commenced. On the basis of the foregoing evidence it was safe to assume that the difference between the quantity of administered glucose and residual glucose found in the intestinal tract after a fixed time interval was the amount of glucose absorbed in that time.

In the present investigation, absorption of glucose was allowed to proceed for 1-, 2-, 3- and 4-hour periods, in 4 respective groups of birds. In addition to the absorption rate, the changes in the blood sugar and glycogen levels were also determined.

The results of these experiments are summarized in table 1.

An examination of the absorption rates for the four absorption periods would indicate a gradual decrease in the rate with time. This trend, however, is not marked and a statistical analysis of the differences in rate between each group indicates a high probability that they could have occurred by chance variation. This is in contrast to the findings of Emslie and Henry (3) who reported a striking decrease in absorption coefficient from 655 for 1.5 hours to 245 for 6 hours, expressed as milligram per 100 grams per hour.

The overall mean absorption rate for all 4 groups listed in table 1 is slightly over 400 mgm. This is more than twice the rate found in rats of similar size (2) and over 4 times the rate reported for dogs by Trimble, Carey and Mad-dock (7).

Although these experiments were not extensive enough to study the effect of the concentration of the administered glucose solution on the rate of absorption it is apparent that the slight differences observed cannot be related to the concentration. For example, the concentrations of glucose administered to groups 1 and 2 were respectively 30 and 43 per cent, while the respective absorption rates for these groups were 436 and 430 mgm. Since, in all of the groups, this was the greatest difference in concentration and the least difference in absorption rate, the conclusion is probably justified that moderate differences in concentration are without effect on the rate.

It is difficult to draw any definite conclusions from the response of the blood sugar to the administered glucose. At the end of 1 hour all of the birds in that group showed a uniformly high blood sugar. The 2-hour group showed an inexplicable drop in the blood sugar level. Since the glucose was being absorbed at the same high rate, it might have been expected that the high level

TABLE 1  
*Absorption of glucose from the intestinal tract*

ABSORPTION TIME	NO. OF BIRDS	LIVER GLYCOGEN	MUSCLE GLYCOGEN	BLOOD GLUCOSE	ABSORPTION COEFFICIENT	PER CENT ABS. GLUC. AS LIVER GLYCOGEN	VOL. AND CONC. OF ADMINISTERED GLUCOSE
		mgm. per cent	mgm. per cent	mgm. per cent			
1. One hour . . . . .	9	1448 $\pm$ 112	915 $\pm$ 54	570 $\pm$ 22	436 $\pm$ 26.6	7.4	5 cc.—30%
2. Two hours . . . . .	8	3494 $\pm$ 296	1150 $\pm$ 57	403 $\pm$ 38	430 $\pm$ 10.7	11.8	5 cc.—43%
3. Three hours . . . . .	14	4597 $\pm$ 175	1344 $\pm$ 115	548 $\pm$ 42	408 $\pm$ 15.3	11.3	10 cc.—35%
4. Four hours . . . . .	13	5862 $\pm$ 223	1306 $\pm$ 118	367 $\pm$ 35	385 $\pm$ 15.4	12.3	10 cc.—40%

of the blood sugar would have been maintained. At the end of 3 hours there was again a rise in the blood sugar, almost to the level of group 1. This was then followed by another drop at the end of the fourth hour. If we attribute the decrease in blood sugar at the end of the second hour to the relatively greater deposition of liver glycogen that took place during that time, the same reason would not apply for the decrease in the blood sugar level at the end of the fourth hour. During this last hour, roughly, the same amount of liver glycogen was deposited as during the third hour, yet in this time the blood sugar fell from a mean level of 524 to 367 mgm. per cent. Probably many of the birds excreted glucose during the absorption period. In the absence of any knowledge of the quantities of sugar excreted and of the amount of sugar burned by these birds, it would be futile to speculate further regarding this fluctuation in the blood sugar curve. In a similar experiment on rats, Cori (8) found that the blood sugar curve reached a maximum at the end of 2 hours and then fell off slightly but consistently each hour for the ensuing 3 hours. Nevertheless, at the end of the fifth hour the blood sugar was still at a higher level than it had attained in 1 hour. In spite of the difference in species, it seems that qualita-

tively, at least, the same type of curve should have been obtained with the chicks.

The rate of deposition of liver glycogen appears to have been greatest during the second hour. Since it has been shown (6) that the fasting control level of liver glycogen is about 350 mgm. per cent, then the quantities of liver glycogen deposited during the first, second, third and fourth hours were in round numbers, 1100, 2000, 1100 and 1200 mgm. per cent, respectively. These quantities, if plotted, would give roughly an "S" type of curve. It is interesting that Cori in the paper cited above also obtained a similar curve for the deposition of liver glycogen, with the chief difference that the third hour was the period of greatest deposition.

Finally, mention should be made of the changes observed in the muscle glycogen level. When it is recalled it has been found that the muscle glycogen of fasted control birds averages 775 mgm. per cent and that of normal fed birds about 1100 mgm. per cent (6), it is apparent from the values in table 1 that only 2 hours were required to replenish the muscle glycogen stores. Within the next hour, the glycogen had increased to supernormal levels and was so maintained during the ensuing hour. It is also of interest to mention that 5 of the individual values for birds in groups 3 and 4 of table 1 ranged from 1810 to 2390 mgm. per cent. As far as we are aware these are the highest muscle glycogen values reported for any avian species and perhaps for mammals also.

An attempt has been made to estimate the amount of absorbed glucose which could be accounted for through glycogen formation. The increase of muscle glycogen observed in the pectoral muscles may have been greater than the increase in the other skeletal muscles. However, if the assumption is made that the observed increase is representative of the entire musculature, then it is possible to arrive at an estimate of the maximum quantity of glucose deposited as muscle glycogen.

A 200 gram chick has about 50 grams of muscle.<sup>2</sup> During 4 hours of glucose absorption the pectoral muscle glycogen increases about 500 mgm. per cent. If this increase is representative for the entire musculature, then a total of 250 mgm. of glycogen would have been deposited. In 4 hours a 200 gram chick will have absorbed 3200 mgm. of glucose (an absorption coeff. of 400). Accordingly a maximum of 7.5 per cent of the absorbed glucose would be accounted for as extra muscle glycogen. Analysis of the liver (table 1) shows that in 4 hours 12.3 per cent of the absorbed glucose is deposited as glycogen and this added to the quantity estimated to be deposited as muscle glycogen shows that not more than 20 per cent of the absorbed sugar can be accounted for in this manner. This is in striking contrast to the 24-hour fasted rat which deposits in the muscle 25 per cent and in the liver 17 per cent of the glucose absorbed in 4 hours (Cori and Cori, 9), a total of 42 per cent, or more than twice the maximum quantity estimated for the chick. While the chick may have a relatively greater amount of glucose distributed in its body water, by far the largest proportion of the absorbed glucose must be accounted for by excretion, oxidation or conversion to

<sup>2</sup> This value was found by direct determinations on another series of birds.

fat. No measure of the excretion could be obtained, but it is extremely unlikely that the glycosuria could have been sufficiently intense to have accounted for very much of the absorbed glucose. Consequently, it would appear that either the chick has a much greater capacity for glucose oxidation than the rat or else is able rapidly to convert a considerable proportion of administered carbohydrate into fat.

#### SUMMARY

The Cori technique has been employed to study the rate of intestinal absorption of glucose in the chick. A rate of 400 mgm. per hour per 100 grams of body weight has been found. This rate appears to remain constant (within experimental error) over a 4-hour period and does not seem to be affected by moderate differences in the concentration of administered glucose. After 4 hours of absorption the liver glycogen had risen to a level of almost 6 per cent while the muscle glycogen rose to about 1300 mgm. per cent. Of the glucose in 4 hours, 12 per cent could be accounted for as increased liver glycogen but not more than 8 per cent was estimated to have been deposited in the muscles. It is suggested that the chick possesses either a relatively intense ability to oxidize glucose or is able to convert a considerable proportion of absorbed glucose into fat.

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## OBSERVATIONS ON THE ACCURACY OF THE THERMOSTROMUHR<sup>1,2</sup>

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Over two and one-half years ago a method was sought to quantitate blood flows in the coronary arteries of normal unanesthetized dogs. While the orifice plate meter (1), and the rotameter (2) were suitable for recording phasic and mean flow in acute experiments, the thermostromuhr seemed the best available device for use in chronic experiments. In attempts to use it, certain difficulties have been encountered which seem insurmountable, and which indicate that under most experimental conditions it cannot be used for quantitative measurements. Since the thermostromuhr is so widely used in just this way, it seems worth while to report our experiences with the method.

**PROCEDURE AND METHOD.** Direct current thermostromuhurs (3) were either bought or constructed according to the specifications of Baldes and Herrick. This type of unit was adopted because of its simple structure. Following standard practice, the units were calibrated *in vitro* before application to arteries in animals. In all uses of the thermostromuhr corrections were made for the null point on the galvanometer before and after each determination. In any one unit, and for a given experiment the heating current was kept constant; in different units this varied from 200 milliamperes to 350 milliamperes. For the calibration, the unit and long, snug-fitting artery segment were connected to an artificial circulation system which produced a flow pattern, as revealed by the orifice plate meter, similar to an aortic pressure curve (cf. fig. 3, part C D-2). The peripheral resistance was never less than 60 mm. Hg, and the calibration was made at room temperature with whole blood of known viscosity (4) rendered non-coagulable with heparin and pontamine fast pink. The actual flow was determined either by a graduate or, more usually, by a calibrated rotameter in series with the unit. The thermostromuhr unit was either placed in a moist chamber<sup>3</sup> or surrounded by saline or cotton saturated with saline.

Following the *in vitro* calibration, the units were applied with aseptic precautions to the arteries of dogs (in most instances the coronary arteries). When the coronaries were used, special precautions, such as the stitching of the unit and its overlying pericardium to the epicardium, minimized movement and angulation of the unit with respect to its artery. Drainage was largely eliminated by periodically packing the lead wire incisions with sulfanilamide.

<sup>1</sup> The expenses of this investigation were defrayed, to a large extent, by a grant from the Commonwealth Fund.

<sup>2</sup> A preliminary report of this work was presented before the American Physiological Society at Chicago, April 15, 1941.

<sup>3</sup> The unit and its contained artery segment were supported in a closed chamber on the floor of which was placed cotton saturated with saline.

Galvanometric readings were taken in 36 dogs (generally on alternate days). Such readings were assumed to represent the same flows as indicated in the *in vitro* calibration. The experiment was generally terminated by calibrating the unit in the above circulation system with the animal's own blood, after careful removal to it of the unit with its artery and surrounding tissue. On occasion, the final calibration was obtained *in situ* by anesthetizing the dog and then connecting a calibrated rotameter peripheral to the unit and varying the flow by means of a screw clamp on the rotameter.

RESULTS. In some of the first experiments a number of days after application of the units, the Bakelite blocks developed electrical leaks and the lead wires frequently broke at the point of emergence from the chest wall. Accordingly, the original braided tinned copper leads were replaced with 8-10 strands of no. 36 or no. 37 annealed silver wire, and the covering varnish or lacquer was replaced by Bakelite lacquer XV-14463. By this procedure electrical and mechanical trouble was largely eliminated in chronic experiments (1-2 mos.).

In some dogs the empirical flow readings derived from the previous *in vitro* calibration were reasonably constant from day to day. In figure 1-A are presented the flow data obtained with a unit that had been on the left circumflex artery of a dog for 30 days (curve *F-2*). After the fifth day the empirical flow varied from 39 to 63 cc./min. Such flows might be the reasonable expectancy in the left circumflex. However, in other dogs such flow figures, although reasonably constant from day to day, vary from 20 to 150 cc./min. in different dogs (12-18 kgm. in weight) (*F-3*, 1). It seems highly improbable that flows in a given coronary branch of different well-trained dogs could vary by so large an amount. Also, since *in vitro* calibrations taken at the beginning and end of a chronic experiment were found to vary considerably (up to 50 per cent) any blood flow values thus derived are questionable. Hence, tests were made to determine the trouble and if possible to eliminate it.

In such tests the unit has been studied in the living dog, and also in an artificial circulation system, to obviate the change of more than one variable at a time.

The first test was the comparison of the *in vivo* and the *in vitro* calibrations of the same thermostromuhr unit. These curves are presented in figure 1-B. For all curves the actual flow was determined by a rotameter inserted peripheral to the unit. For curve 1 the unit was applied to the carotid of an anesthetized dog and the incision closed. The peripheral end of the rotameter was connected to the central end of the jugular vein on the opposite side, and the flow was varied by a screw clamp peripheral to the rotameter. For curve 2 (taken about 15 min. later) an *in vivo* moist chamber was created without disturbing the artery, by careful removal of all tissue and fluid from contact with the artery and by closing the incision with skin and moist towels. For curve 3 (about 30 min. later) the same artery (12 cm. long) with its attached unit was removed to the moist chamber of a circulation system, connected to two 18 inch plastic cannulae without apparent constrictions or deformations, and then calibrated with the same dog's blood at room temperature. These curves differ by about 50 per cent. Obviously, the use of a calibration obtained in a moist chamber is restricted and may not be used to derive flow values in animals.

The next step was the investigation of the cause for the difference between the calibration curves obtained in the animal (curves 1, 2; fig. 1-B). Accordingly, the effects of tissue components *per se* together with the movements of extra-vascular and intra-vascular fluid upon the calibration of the thermostromuhr were tested.

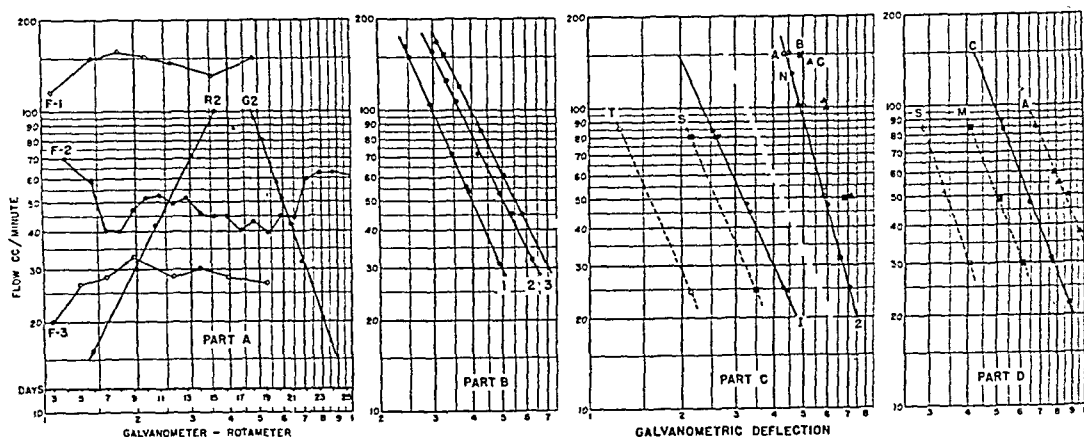


Fig. 1. Part A. Graphs showing blood flow values in left coronary arteries of different dogs calculated from *in vitro* calibration curves of thermostromuhr. *F-1*, *F-2*, *F-3*, empirical flow values in dogs (D-48, 46, 49) weighing 18.0, 12.0 and 12.0 kgm. respectively. *F-1* is based on *G-2* (*in vitro* thermostromuhr calibration) and *R-2* (calibration of rotameter used to calibrate thermostromuhr). Ordinate—coronary flow cc./min. Abscissa—galvanometric deflection in 100 mm. Rotameter—in millimeters. Days—as indicated.

Part B. Curves showing difference between calibrations of thermostromuhr in a moist chamber and *in vivo*. Curve 1, unit on carotid artery of anesthetized dog and surrounding skin and muscle in contact with unit. Curve 2, same set up 15 minutes later, except that *in vivo* "moist chamber" created. Curve 3, unit and artery of curve 2 carefully removed to pump system and calibrated at room temperature with same dog's blood. For all curves flow through unit measured by rotameter connected peripheral to it.

Part C. *In vitro* calibration curves obtained on same thermostromuhr unit with different environmental conditions prevailing. Curve 1, obtained on unit after it had been applied to femoral artery of dog for two weeks and then removed to calibration system with its contained artery and surrounding tissue. Points *S*—same but after artery stretched about 10 per cent. Points *T*—same but tissue carefully clipped from unit and artery. Curve 2—unit calibrated in a pump system surrounded by saline, *N* (dots); ascitic fluid *A* (open circles); whole blood, *B* (squares); blood diluted 1:3, *C* (triangles).

Part D. Unit calibrated in pump system and surrounded successively by saline soaked cotton, *C*; fresh muscle tissue, *M*; saline, *S*; moist chamber, *A*.

*Effects of environmental changes.* In an artificial circulation system maintained at room temperature, the calibration curves are essentially identical when saline or ascitic fluid surround the unit, but if the surrounding fluid is more viscous (whole blood or blood diluted 1:3), shifts of 50 to 100 per cent in the calibration curve occur (fig. 1-C, curve 2, points *B*, *C*). If a unit with its artery segment is surrounded by cotton soaked in saline, and then in succession by fresh moist tissue, saline alone, and finally, a moist chamber, curve *C* shifts to curves *M*, *S* and *A* respectively (fig. 1-D).

To test further the "tissue effect" in animals, units have been applied to the femoral arteries of a number of normal dogs for 2 weeks, and then the unit and artery, together with the surrounding tissue, have been removed to the calibration system. Curve 1, figure 1-C, was obtained from such a unit. After tissue removal from the region of the unit by careful dissection, calibration gives points *T*. Similar shifts in the calibration curve have been obtained following tissue removal from the unit applied to a femoral artery for two weeks and then calibrated *in situ*.

*Movements of intra-vascular fluid.* A thermostromuhr is directly affected by the flow of blood in the capillary bed and large vessels around it, as indicated by changes in galvanometer deflection without concurrent changes in blood flow through the unit. Representative data in figure 2-A have been taken from different experiments and arranged so that the same apparent shifts in flow occur as in each experiment. To obtain the data, the unit and its contained excised artery were buried between muscle layers of an anesthetized dog and then connected with the calibration system and rotameter, the whole ensemble being maintained at essentially constant temperature in a water bath. The flow through the calibration system and unit was maintained constant, as indicated by the rotameter, and flow alterations in the surrounding tissue were induced progressively by mechanical constriction of its nutrient artery (iliac) or by vasodilator or constrictor drugs. The apparent flow as read from the galvanometer deflection shifts about 30 per cent (dots and circle) while the actual flow through the unit is unchanged.

Similarly, if in the same arrangement the flow through the unit is again kept constant and the cold junction of the unit is purposely placed close to the femoral artery of the host dog, reduction of flow through this same artery causes shifts in the galvanometric deflection (triangles) indicating apparent flow increases of 40 to 300 per cent through the unit, despite the constant reading of the rotameter. Similarly, if the area over the hot junction is placed in contact with the femoral artery of the anesthetized dog and blood flow through that artery is reduced, errors of comparable magnitude, (squares) but in the reverse direction, are noted. The same results can be obtained in a calibration system if the rate of fluid flow is changed through a small rubber tube placed in the same fluid bath as the unit, and close to the latter.

*Movements of extravascular fluid.* Such movements can also significantly affect the calibration curve. Ten to fourteen days after a unit had been applied to the iliac artery of a dog, the dog was anesthetized and the unit area exposed and observed. Visible fluid movements about the unit induced by intestinal activity resulted in marked alterations of the galvanometric deflection. Similar results were obtained in a calibration system by stirring or tilting the chamber fluid.

While the variations in external environment adequately explain the differences between curves 1 and 2 in figure 1-B, to account for the difference between the moist chamber calibration in an artificial circulation system (curve 3) and the *in vivo* calibration (curve 1), additional factors such as differences in



heart rate, stroke volume, viscosity, pattern of the flow curve, in the vessels used and in their contact with the thermostromuhr unit must be considered. Some of these variables are quite effective, others are not.

*Temperature, heart rate, and stroke volume.* These are potentially effective variables which have little or no effect on the accuracy of the thermostromuhr unit. In figure 2-B the calibration curves taken in an artificial circulation system indicate that variations in blood temperature of  $\pm 4^{\circ}\text{C}$  (curve 1), alterations of

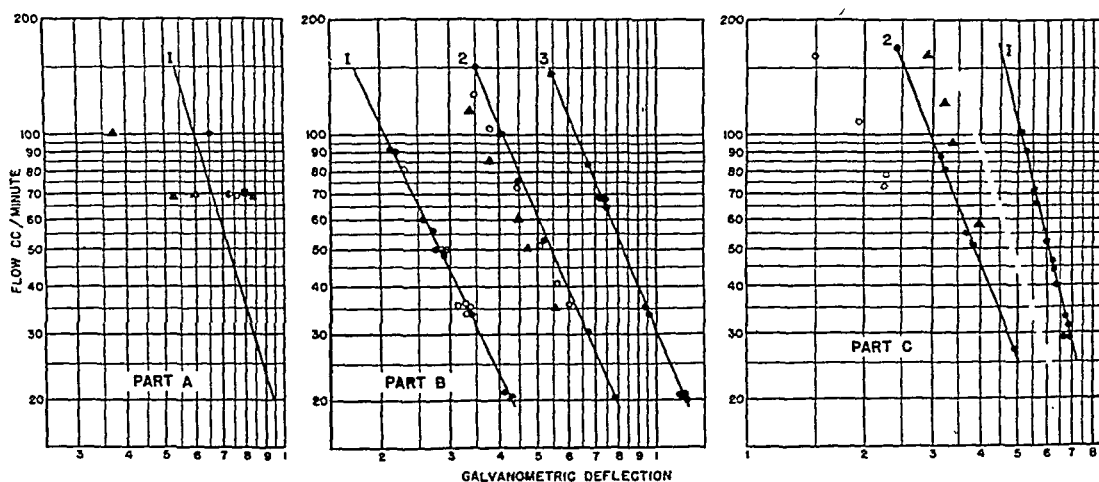


Fig. 2. Part A. Effect of change of external environment in living animal on calibration curve of thermostromuhr. Curve 1, assumed calibration curve from which recorded points are placed so that they show same percentage error as in original experiments. Unit and contained artery segment embedded between muscle layers in leg of anesthetized dog and connected to pump system. Dots, iliac artery partially constricted. Circle, nitroglycerine injected by vein. Triangles, cold junction of unit placed near femoral artery with iliac artery flow reduced mechanically. Squares, same but with hot junction near femoral artery.

Part B. Effects of alteration of temperature, (curve 1), viscosity (curve 2), and heart rate and stroke volume (curve 3) on calibration curves obtained in a moist chamber. Dots—control points. Temperature change from  $29^{\circ}\text{C}$  (dots) to  $24^{\circ}\text{C}$  (circles). Specific viscosity change from 8.4 (dots) to 5.3 (circles) to 3.1 (triangles).

Part C. Curve 1, calibration points obtained with blood at room temperature over a four-hour period. Thermostromuhr surrounded by saline in an artificial circulation system. Curve 2, calibration of unit in moist chamber showing effects of angulation and movement of unit on its contained artery. Dots, control points. Circles, angulation. Triangles, unit movements.

heart rate (pump rate) from 90 to 300 per minute, and gross changes in the pump stroke (curve 3) do not materially affect the calibration curve.

*Blood viscosity.* The specific viscosity of blood in normal, unanesthetized dogs, as determined in a previous investigation (4), varies from 3.7 to 7.0, with most of the values falling between 4.0 and 6.0. When the specific viscosity in the circulation system is altered from 8.4 to 2.8 by adding or subtracting red cells or plasma or by adding saline, the calibration shifts about 25 per cent (cf. fig. 2-B, curve 2).

*Differences in vessel and contact.* Various *in vitro* calibrations of the thermostromuhr unit on the same artery in a calibration system can agree very closely, provided internal and external conditions are unchanged. For example, in figure 2-C, curve 1, with the unit and artery suspended in a moist chamber, the maximal error from the initial calibration curve over a four hour period is 10 per cent.

However, calibration of the same unit applied to different arteries in an artificial circulation system may give calibration curves of considerable difference, all other conditions remaining the same. In figure 3-A, the extremes of three

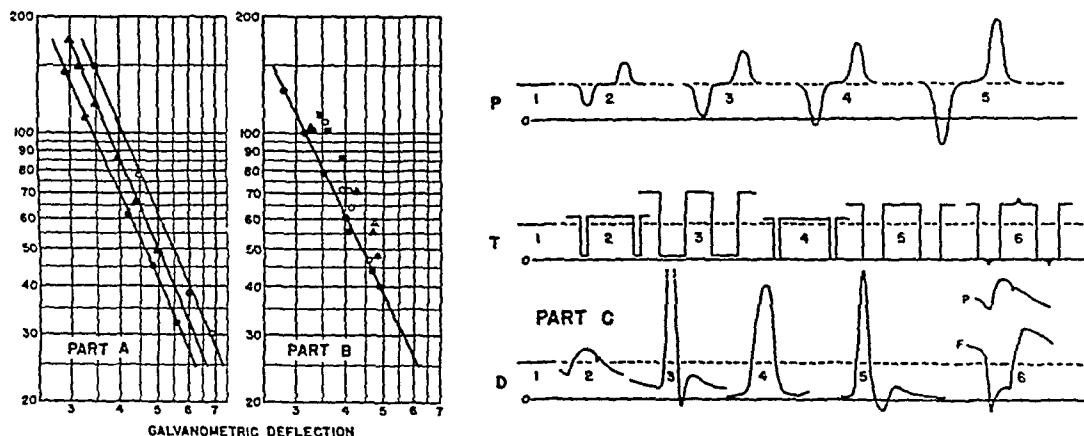


Fig. 3. Part A. Calibration curves obtained with blood at room temperature on same thermostromuhr unit applied to three different arteries in a moist chamber of a circulation system.

Part B. Calibration curve on carotid artery of anesthetized dog with unit surrounded by its normal complement of tissue and fluid. Unit calibrated by rotameter placed peripherally and connected to central end of opposite jugular vein. Dots—initial control points. Squares—aminophylline injection. Triangles—nitroglycerine injection. Circles—control points after drugs.

Part C. Diagrams illustrating various flow patterns produced by a pump system and recorded by orifice plate meter. D-2, 3, 4, 5 and 6, flow patterns similar to those existing in carotid, femoral, coronary sinus, femoral, and left coronary respectively, in anesthetized dogs. P, aortic pressure. F, flow. O, zero flow.

typical calibration curves obtained in a moist chamber on the same unit vary by about 40 per cent.

Similarly, movements of the thermostromuhr unit relative to the artery can significantly affect the calibration curve. For the data in figure 2-C, curve 2, the unit was placed on a 12 cm. length of carotid artery in a moist chamber and calibrated. Readings were then made, with the unit placed in different positions along the distal half of the artery, with the artery held at different lengths, or with the unit angulated 20 to 30° with respect to the long axis of the artery. The errors thus induced vary from 22 to 100 per cent. It should be emphasized that movements of the unit on the same artery and the use of different arteries may not always influence the calibration curve.

Similar errors occurred in experiments in which the unit was calibrated against

a rotameter in an anesthetized dog. Attempts to make the contact more permanent by long continued application of the unit to an artery of a dog have proved unsuccessful. Units have been placed on the femoral arteries of dogs for two weeks and then calibrated *in vivo* with a rotameter. Slight changes (10 per cent or so) in the length of the artery (manually induced) give points *S* (30 per cent shift) as compared to control curve 1, in figure 1-C.

*Pattern of the flow curve.* As demonstrated by the orifice plate meter, the contour of the flow curve in various arteries of the anesthetized dog is different. In the right coronary (5) the flow is presumably always forward, although phasic velocity varies greatly. In the left coronary (1, 7) and the femoral artery (6) of the anesthetized dog, and in the human femoral (8) the presence of zero flow and back flow components is the usual finding, while in the carotid and numerous other arteries these flow components can appear. Such phasic alterations in velocity and direction of flow could conceivably affect the accuracy of the unit. This has been tested in the circulation system and in the anesthetized dog.

In a circulation system, the flow curves, as determined by an optically recording orifice plate meter, interposed distal and close to the unit, range from those of linear conformation, high and low velocity components, those with zero and back flows to those simulating a left coronary, carotid, femoral artery and coronary sinus pattern (for sample curves cf fig. 3, part C, *D*).<sup>4</sup> In general, phasic variations of flow velocity during a mechanical cycle do not grossly affect the calibration curve, provided the rate of flow never approaches zero (cf. fig. 3, part C, *P-1*, 2, 3; *T-1*, 2; *D-1*, 2). However, if the velocity is relatively low for a considerable portion of the cycle (*T-3*) and particularly if the fast and slow components of the cycle show great differences, as in *D-4*, the galvanometer indicates a somewhat greater flow (fig. 4-A) (circles) than is actually passing through the unit. Introduction of periods of zero flow existing for 10 per cent or more of the cycle (fig 3, part C, *T-4*, 5) reduces the galvanometric deflection considerably, and the error is roughly of the order of 1 per cent for each percent of zero flow in cycle time (fig. 4-A, dots).

When small back flows (2-10 per cent of cycle volume) of short duration (2-10 per cent of cycle time), as in figure 3, part C, *P-4*, are induced or are superimposed on an already existing period of zero flow (fig. 3, part C, *T-6*) the error can be 50 per cent (fig. 4-B). Larger back flows spaced in the same or a slightly longer time period (fig. 3, part C, *P-5*) give larger errors. However, the most striking effects have been seen in flow patterns with small back flow volumes spread over a considerable portion of the cycle. In figure 4-B, the circles, connected by interrupted lines, were obtained with flow patterns in which the portion of the cycle occupied by back flow was about 1 to 30 per cent in cycle time, and less than 10 per cent in cycle volume. The error reaches a few hundred percent. In "synthetic" coronary flow curves (fig. 3, part C, *D-6*) taken from different experiments and with back flows of 3 to 10 per cent of cycle

<sup>4</sup> The flow curves represented were created by a combination of various expedients, the description of which is beyond the scope of this paper.

volume and occupying 3 to 10 per cent of cycle time, the apparent increase in flow varies from 45 to 95 per cent (fig. 4-B).

Similarly, in the anesthetized dog, flow measurements by means of the thermostromuhr on arteries in which back flow is known to exist are attended by large errors. In figure 4, part C, are shown typical results with use on the femoral artery before (point C) and during (circles) late stages of the effect of the injection of a small amount of epinephrine (jugular vein) as contrasted with the control-curve without back flow (dots and uninterrupted line) obtained by

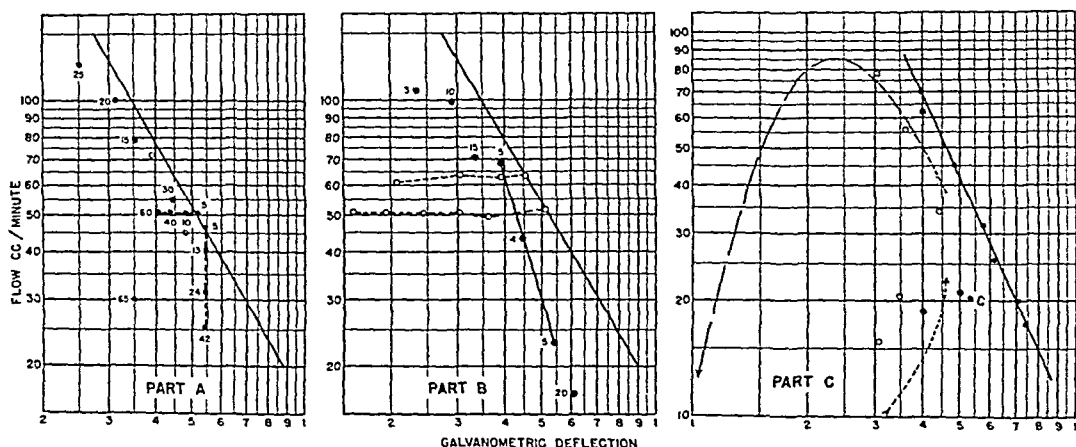


Fig. 4. Part A. Effect of near zero and zero flow on calibration curve of thermostromuhr. Points are from different experiments and curve is assumed calibration from which recorded points are placed so that same percentage error exists as in original experiments. Numbers indicate percent zero flow. Connected points are from same experiment.

Part B. Effect of back flow on thermostromuhr calibration. Long curve—same as in part A. Numbers indicate percent back flow volume. For all dots, back flow occupied from 2 to 10 per cent cycle time. Connected points are from same experiment. Circles, approximately 2 to 10 per cent back flow volume spread over 1 to 30 per cent of cycle time.

Part C. Graph showing observations made with thermostromuhr applied to femoral artery of anesthetized dog. Dots, control points when flow (adjusted by screw clamp) is from femoral artery to femoral vein of same leg and flow pattern contains no zero or back flow. C, normal flow through femoral artery containing periods of zero flow and back flow. Circles, points after zero and back flow increased by injection of epinephrine (0.07 mgm.) by jugular vein. Dotted line, trend of galvanometric deflection and blood flow after epinephrine injection.

shunting femoral artery flow into its femoral vein. (Pattern and actual flow determined by orifice plate meter and rotameter.) A more complete picture (based on a number of experiments) of the trend of the temporal relations of actual flow and galvanometric deflection (interrupted line) throughout the effects of epinephrine injection is further instructive. While the actual flow increases and then decreases, the galvanometric deflection decreases only, and its direction actually reverses with relation to flow. This is manifest by reversal of the slope of the curve. Orifice plate meter records indicate that back flow present before injection diminishes near the beginning of the flow increase, is augmented as flow begins to drop, and becomes maximal at lower flow levels.

This means that the effect of the augmentation of back flow has exceeded the effects of decreasing flow, so that the galvanometer registers a flow increase, when actually a flow decrease exists.

Finally, in the use of the thermostromuhr another potential error exists, which is not revealed by a calibration curve. This is the considerable mechanical limitation of flow by the thermostromuhr *per se* (9).

DISCUSSION. Various types of thermostromuhrs are used to quantitate blood flows. The differential temperature of the two thermocouples, as in-

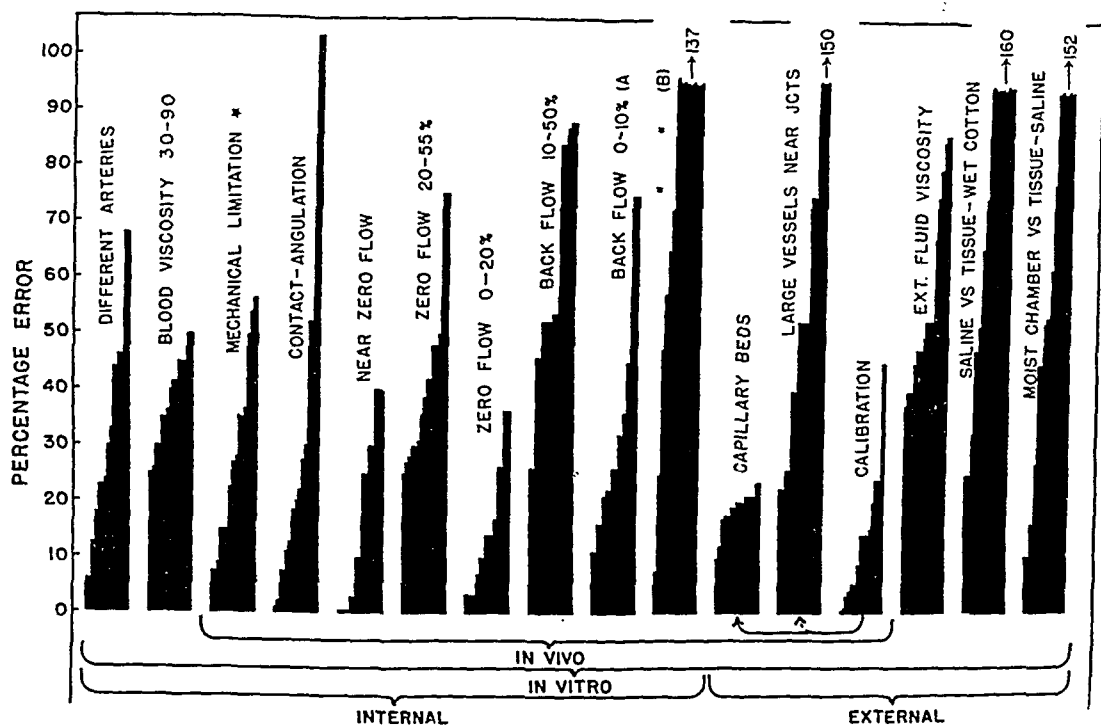


Fig. 5. Summary chart illustrating the extent of errors of internal and external environmental origin which were found to exist when thermostromuhr was calibrated *in vivo* and *in vitro*. Each bar segment represents two or more observations. (A) Back flow 1 to 10 per cent by volume occupying 1 to 10 per cent of cycle time. (B) Back flow 1 to 10 per cent by volume occupying 10 to 30 per cent of cycle time. \* Data on effect of mechanical limitation of flow taken from paper of Shipley and Gregg (9).

indicated by the galvanometer, has been assumed to be an accurate index of flow, once the relation has been established by calibration. This assumes that essentially all, or a constant percentage of the heat from the heater enters the blood stream, and that at any one flow no internal or external influence affects this relation. On this basis an *in vitro* calibration of the thermostromuhr should give the correct blood flow values for galvanometric deflections obtained from the same unit applied to a vessel of a living animal.

The data presented here do not substantiate this assumption, but rather indicate that the differential temperature of the couples reflects changes in the internal and external environment of the unit other than those induced by

changes in rate of blood flow alone. For convenience, the percentage deviation of the empirical flow from the actual flow that can be induced by these variables is summarized in figure 5. Among such effective influences are 1, the position of the unit on the artery and its degree of angulation with respect to its contained artery; 2, use of different arteries; 3, blood viscosity; 4, the existence of near zero, zero and back flows in the pattern of the flow curve; 5, character of fluid and material around the unit block, and 6, the flow of extravascular fluid and of blood in the capillary beds and larger vessels near the thermostromuhr. In addition, the thermostromuhr mechanically limits flow through itself, presumably without causing any changes in the calibration curve.

The effects of these different factors may be sizable. Separately, the errors range from 0 to 300 per cent; taken together, the error is fantastic. However, it should be emphasized that in many calibrations, some of the errors will not exist, and the direction and magnitude of the individual error will vary with the individual unit and dynamic conditions prevailing.

*In vitro* calibrations on the same unit are found to vary with the external environment, and all generally differ from an *in vivo* calibration. Therefore, the flow of blood in an artery of an animal can only by chance happening be determined from a unit applied to it which had been previously calibrated in an artificial circulation system. This is so, because, for the same unit, it is indeed an accident when *in vitro* and *in vivo* environments influence the differential temperature-flow relations in the same direction and to the same extent. It is difficult to understand the contrary viewpoint of some (10, 11) that the practice of obtaining a calibration curve on any vessel in a moist chamber and pump system and applying it to readings made with the unit placed on an artery in a normal, unanesthetized dog permits errors of not more than 10 per cent. The experimental proof for such a view has not yet appeared. Such a view ignores the observations of Baldes (3) that maximal and minimal values in calibration curves for the same unit on different arteries vary by as much as 50 per cent, the observations of Grodins, Osborne, Ivy and Goldman (12) that *in vitro* calibrations on the same unit disagree by sizable amounts from *in vivo* calibrations on the hepatic artery, and, finally, the potent and unpredictable effects on a calibration curve demonstrated here of changes in the external and internal environment.

Our data indicate that the differential temperature of the thermocouples of the thermostromuhr fails to retain its calibrated relation to flow in the presence of flow patterns containing periods of near zero, zero, or back flow, regardless of the application of a unit to *in vitro* or *in vivo* experiments. In the case of back flow the error is many times greater than a simple back flow/total flow ratio would indicate. Since, in acute and chronic experiments, the volume and timing of these flow components change with dynamic conditions, and such patterns cannot be predicted and duplicated in a calibration system, calibration curves with quantitative significance cannot be obtained for units when applied to such arteries either *in vitro* or *in vivo*.

In animal preparations it is also possible that the galvanometric deflection

will not always decrease as the flow increases, and vice versa. In the femoral artery of the anesthetized dog in which back flow has been shown to occur under experimental conditions, injection of a constrictor drug may so augment the back flow that the direction of the galvanometric deflection will be completely reversed with respect to flow. Similarly, instances have occurred in which, after a unit had been applied to a coronary artery for many days, empirical flow values of reasonable expectancy have been recorded which increase upon injection of epinephrine. Yet, autopsy an hour later revealed that the artery had been completely occluded, apparently for some days. In short experiments (a few hours), however, and under most circumstances, the thermostromuhr will presumably indicate correctly directional changes in flow, provided the blood flow is at all times forward, and at no time approaches zero.

*In vivo* calibration of the unit should remove or minimize some of the errors associated with its use on arteries exhibiting all forward flow, in either acute or chronic experiments. To test this, the units were applied to carotid arteries of anesthetized dogs and the blood flow measured by a calibrated rotameter connected to the artery peripheral to the unit. Since back flow can occur in carotid flow patterns an all forward flow was made certain in these experiments by shunting blood flow (controlled by a screw clamp) from the peripheral end of the carotid to the central end of the severed jugular vein on the opposite side. After the incision was closed and a calibration curve obtained on the unit, the carotid flow and galvanometric deflections were recorded for a period of from 1 to 3 hours. At times during this period the flow was altered by various means, such as drug injection. Any deviation of the galvanometric deflection from the known flow through the unit on the basis of the preliminary calibration curve gives the extent of error due to the change in environment. A typical curve presented in figure 3-B indicates the expected error may be sizable, and varies from 0 to 40 per cent. In long continued experiments (days to weeks) the error of such an *in vivo* calibration would be expected to be much greater because of the unknown amounts of angulation, mechanical reduction of flow by the unit (in many instances enhanced due to thickening of the artery wall), alterations in the external environment of the unit occurring naturally, or that also presumably induced by the anesthetic and anticoagulant used for final calibration, and, finally, possible changes in flow in the vasa vasorum.<sup>5</sup>

The fact that the *in vivo* and *in vitro* calibration curves for any one unit are approximately parallel, although not superimposable, suggests that percentage changes in flow in an experiment can be determined from any one of these curves with fair accuracy. The prerequisite is that in an actual experiment the en-

<sup>5</sup> This is suggested by the observation that the apparent blood flow through a thermostromuhr on a carotid artery in an anesthetized dog may shift appreciably with drug injections, despite the facts that the unit is without contact with the surrounding tissue and fluid ("moist chamber"), any preexisting back flow or zero flow is eliminated from the flow pattern by the procedure described above as used for obtaining the data for fig. 3-B, the artery central to the unit is uncut and the actual flow through the unit is measured by a rotameter and kept constant by a screw clamp on the latter.

vironmental conditions and pattern of the flow curve are as well stabilized as when under the rigidly controlled conditions for calibration. Presumably, this state is approached in experiments in which flow in a vessel is at all times forward, and circulatory conditions in the animal are undisturbed. However, it is difficult to conceive of changes in blood flow through a vessel, resulting from drug injection, alterations of blood pressure, heart rate, etc., without simultaneous changes in the immediate environment and/or flow pattern, with their attendant large effects on the calibration curve (cf. fig. 4, part C). Since these changes necessitate the establishment of a new equilibrium, the true calibration curve no longer remains fixed, but may be actively shifting either to left or right, coincident with, but not caused by the flow change in the vessel. Thus, the true moment to moment relation of flow to galvanometric deflection which is applicable to a given series of flow changes may no longer be straight or parallel to any previously or subsequently obtained calibration curve. Hence, the percentage change in flow cannot be determined.

#### SUMMARY AND CONCLUSIONS

Direct current thermostromuhrs, with improved lacquers and lead wires, have been applied to the arteries of dogs in acute and chronic experiments. The units have been calibrated in an artificial circulation system, or *in situ* by means of a rotameter. Tests made in the circulation system and in the anesthetized dog indicate that blood flow values in chronic experiments, as read from an *in vivo* or *in vitro* calibration, may be highly inaccurate because the relation of the galvanometric deflection to flow will vary with 1, the artery used, and its degree of stretch; 2, the position and degree of angulation of the unit with respect to its contained artery; 3, the presence or absence of near zero, zero or back flow in the flow pattern of the metered fluid; 4, composition of the immediate environment; 5, movements of extra- and intra-vascular fluid in the environment, and 6, viscosity of the metered fluid.

In acute experiments application of *in vitro* calibrations is not justified for the same reasons. *In vivo* calibrations may allow a semi-quantitative measure of flow provided environmental influences are eliminated and flow is at all times forward.

Since, in any one experiment, the occurrence and magnitude of the effect of most variables can neither be controlled nor predicted, the conclusion is reached that any interpretation of galvanometric deflections obtained with a thermostromuhr in acute or chronic experiments in terms of absolute blood flow or percentage change in flow is open to grave question.

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# OPERATIVE MECHANISM OF SOME ERRORS IN THE APPLICATION OF THE THERMOSTROMUHR METHOD TO THE MEASUREMENT OF BLOOD FLOW<sup>1,2</sup>

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In a preceding paper (1) a practical evaluation of the accuracy of the thermostromuhr (Baldes and Herrick type) has revealed that, by changes of environment and flow pattern, sizable errors are introduced into its quantitation of blood flow. To clarify the *modus operandi* of these factors the mechanism whereby changes in galvanometric deflection reflect changes in flow was studied.

Since, with a thermostromuhr of this type, the galvanometric deflection varies with the differential temperature of the two thermocouples, but the amounts of deflection and flow vary in opposite directions, it is logically concluded that the higher the flow the less temperature difference exists between the two thermocouples. To evaluate the contribution of individual couple temperatures to this differential temperature a special thermostromuhr unit was constructed for this and other studies.

In substitution for the ultimate use of a blood vessel a thin-walled glass tube 12 cm. in length (O.D.—2.23 mm.; I.D.—2.10 mm.) was permanently built into a conventionally shaped unit (10 mm. broad, 10 mm. long, and 3.5 mm. thick). The heater and couple elements were held in contact with the outside of the tube during the hardening of the lacquer. The Bakelite block (BT48-005) and lacquer (BL-3128) used gave essentially a transparent unit. In addition, the wiring was modified to the following extent: The constantan connection between couples within the block was eliminated by running the constantan wires directly through the block to the outside, where connections could be made for recording the temperature of each junction separately. Elimination of this connection removed a potential pathway for direct heat transfer to the couples from the heater area. Also, the couples were placed equidistant from the flat sides of the unit on the floor of the lumen where they would presumably be least vulnerable to environmental influences. A reference thermocouple placed in a thermos bottle completed the galvanometer circuit.

The unit, surrounded by dry cotton, was connected in a pump system and perfused with acacia solution (spec. visc. 4.0—4.5) at room temperature. The sensitivity of each couple was determined with reference to the thermocouple in the thermos bottle.

<sup>1</sup> The expenses of this investigation were defrayed to a large extent by a grant from the Commonwealth Fund.

<sup>2</sup> Preliminary reports of this work were presented before the American Physiological Society at the Boston Meeting, April 1942 and before the Cleveland Section of the Society for Experimental Biology and Medicine December 12, 1941.

In figure 1-B are shown the elevations in temperature [above that of the perfusing fluid and environment (room temperature)] at the two thermocouples with three heating intensities and at several rates of flow through the unit. Also shown are what could be called calibration curves representing the differential temperatures computed by subtraction. In figure 1-A are plotted corresponding temperatures of the perfusion fluid opposite the two couples as determined by exploring thermocouples inserted in the glass tube to the level of the standard couples and in contact with the wall immediately over each.

For purposes of revealing the significance of these curves, let us assume that a hypothetical state exists within a unit in which the cold or upstream junction

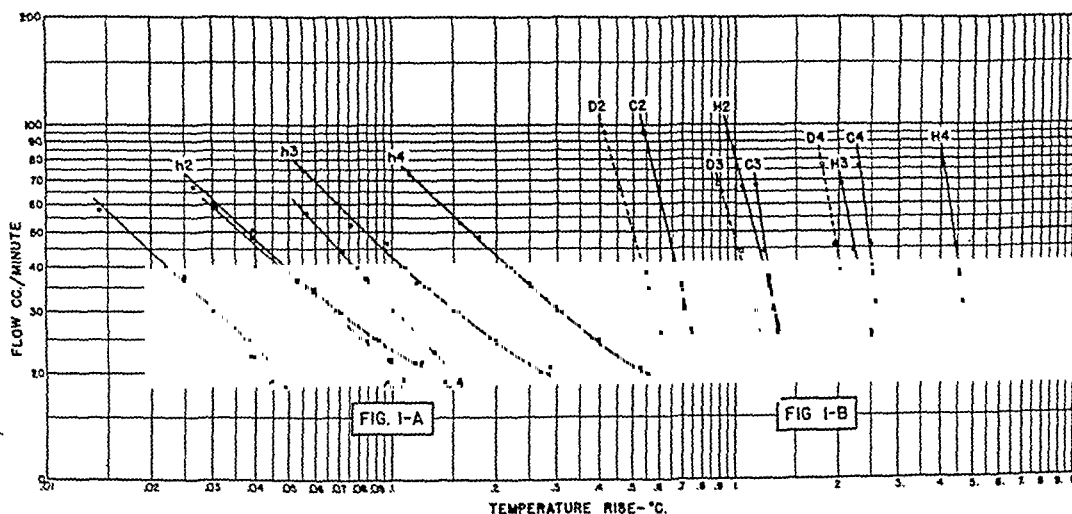


Fig. 1. A. Curves indicating temperatures of fluid (above that of incoming fluid) measured immediately opposite the hot, *h*, and cold, *c*, thermocouples when thermostromuhr unit was perfused with acacia solution (spec. visc. 4.0-4.5) at room temperature with heater currents of 200 (2), 300 (3), and 400 (4) milliamperes. Ordinate: Flow in cc./min. Abscissa: temperature rise °C.

B. Curves of individual thermocouple temperatures of same thermostromuhr with differential temperatures at different flows and heating intensities. *H*, hot thermocouple; *C* cold thermocouple; *D*, differential temperature. 2, 3, and 4—same as in figure 1-A. Ordinate and abscissa—same as in figure 1-A.

and incoming fluid are of the same temperature, and the hot or downstream junction faithfully records all, or a constant proportion of the temperature rise sustained by the fluid as it passes the heater. The differential temperatures obtained would show that as the flow doubled the deflection would decrease by one half, thereby affording maximal "sensitivity." That such optimal conditions do not exist in ordinary practice is at once apparent (fig. 1-A, 1-B). First, the temperature of the cold junction is not the same as that of the incoming fluid. Second, the temperature of the hot junction does not vary inversely as the flow, and therefore is not entirely influenced by the rate of flow alone. And third, the temperature of each junction is considerably in excess of the temperature of the fluid passing by each ( $C_2$  versus  $c_2$ ,  $H_3$  versus  $h_3$ , etc.). The concept

of a conceivable working distribution of heat within a thin sagittal section is diagrammed in figure 2.

It would seem, therefore, that the great bulk of heat reaching both of the unit's thermocouples is supplied directly via the block. And since both couples are hotter than the passing fluid it follows that changes in the rate of flow alter the differential temperature through corresponding changes in the rate of *cooling* of both couples. The extent of cooling depends primarily upon two interdependent factors: first, how hot the couple is (determined by the rate heat is supplied from the block and the rate at which it is removed by the fluid) and, second, how cool the fluid is (determined by the temperature of the fluid enter-

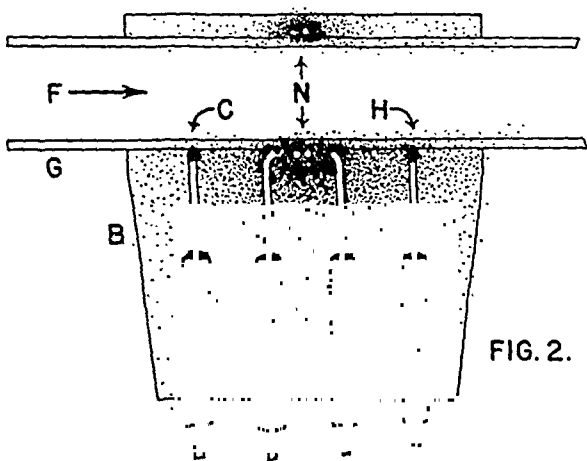


FIG. 2.

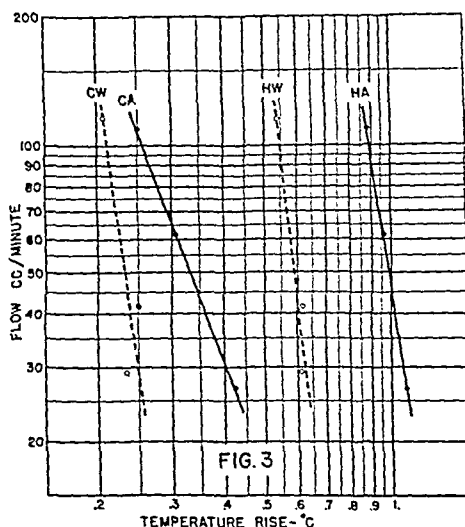


FIG. 3

Fig. 2. Diagram of concept of gross heat distribution within a thin sagittal section of thermostromuhr described in text. Density of stippling indicates relative temperature elevation. *F*, fluid; *G*, glass tube; *B*, Bakelite block; *C*, cold junction; *H*, hot junction; *N*, heater.

Fig. 3. Curves showing temperature rise of thermocouples applied to exterior of thermostromuhr unit over the respective sites of the internal thermocouples with external environments of air and of water. *H*, thermocouple over hot junction; *C*, thermocouple over cold junction; *A*, temperature curves with unit surrounded by air (dry cotton). *W*, unit surrounded by water (water saturated cotton). Ordinate and abscissa—same as in figure 1.

ing the unit and the temperature rise sustained as it approaches the couple area).

In general, the heat gradient through the block extends centrifugally, with the heater its highest point (fig. 2). During flow the axial ends of the unit with their contained thermocouples, even though carefully placed equidistant from the heater, will not have the same temperatures because of the greater cooling effect of the unheated incoming fluid upon the upstream end. The extent to which the temperature of the fluid opposite each junction influences the respective junction temperatures with a given change in flow is indicated by the different slopes of the junction temperature curves at any given heating intensity ( $C_2$  versus  $H_2$ , etc.) (fig. 1-B).

The foregoing considerations indicate that a calibration curve represents the relation of the rate of flow to the differential temperature of two thermocouples whose individual temperatures are determined by the respective *rates at which they are cooled* which, in turn, are derived from the heat gradients between the heater and fluid stream at the sites of the thermocouples.

*Environmental influences, externally induced.* The above study demonstrates that the influence of the heater extends considerably beyond its immediate environment. A sizable temperature elevation was observed at the upstream junction, in spite of its proximity to the incoming fluid (fig. 1-A). This led to the belief that a variable amount of heat would be lost to the outside environment through the block, and thereby alter the thermal equilibria at the two junctions. Accordingly, two thermocouples were lacquered to the outside of the block immediately over the sites of the internal couples. After calibration of the couples local temperatures were then recorded under rigidly controlled conditions with the unit surrounded first by a layer of dry cotton and then by water saturated cotton, at room temperature. In figure 3 are plotted the observed elevations in temperature (above room temperature) of the two outside thermocouples at different rates of flow. The change in the environment from air to water permitted a significant drop in the external junction temperatures indicating greater heat transfer to the environment.

To estimate the total extent of heat loss, the fractional transfer of heat to the fluid passing through the unit and to the surrounding medium was roughly quantitated. The heat generated within the unit was calculated according to Joule's law from the equation  $0.239I^2R = \text{Cal/Gm/Sec.}$  in which  $I = 0.2, 0.3$  and  $0.4$ , and  $R = 3.95, 4.15$  and  $4.28$ , respectively. The temperature rise of the fluid was determined by an exploring thermocouple fixed in the center of a plastic tube (inside diameter—1 mm.) and located 1 mm. from the tip. This tube was then inserted from the downstream end into the glass tube running through the unit to a point 29 mm. from the downstream edge of the unit. The intervening length of glass tube was packed with glass wool (to insure thorough fluid mixing) and surrounded with layers of tinfoil and dry cotton to minimize heat loss. After calibration of the exploring couple, distilled water was run through the unit at various rates and the corresponding temperature rises observed. The heat loss to the environment was estimated indirectly by subtracting the heat uptake by the fluid from that generated by the heater.

Since Cal/Gm/Sec. at 60 cc./min. is numerically equal to the temperature rise in  $^{\circ}\text{C}$  at that flow and the temperature rise of the fluid should vary inversely as the flow, a theoretical temperature for any flow may be plotted. Undoubtedly a small heat loss occurred from the point at which the fluid left the unit to that of recording. However, such losses are negligible compared to those sustained to the external environment of the unit during its operation.

In figure 4 are shown points representing a rough quantitation of the heat transfer to the fluid passing through the unit at different rates of flow and at three applied heating intensities. The extent of heat transfer is represented in terms of temperature elevation above that of the incoming fluid (room tem-

perature). The interval separating the points from the curves (indicating theoretically optimal or 100 per cent transfer) represents the degree of heat loss via the block.

While the heat losses to an environment of quiescent air (dry cotton) are detectable but small, those to water saturated cotton are sizable, averaging 25 to 30 per cent over all ranges. Similarly, an environment of air with 100 per cent water saturation ("moist chamber") in contrast with that of muscle tissue yielded results (points not shown) which were indistinguishable from those obtained above with room air versus water. Such differences could be expected considering the different abilities of these substances to take up heat per unit volume (water = 1 Cal/cc./°C rise, air = 0.0003 Cal/cc./°C rise).

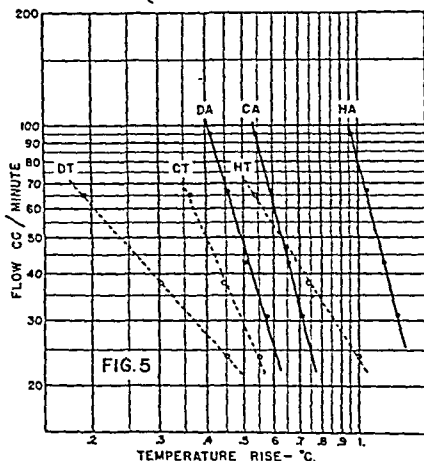
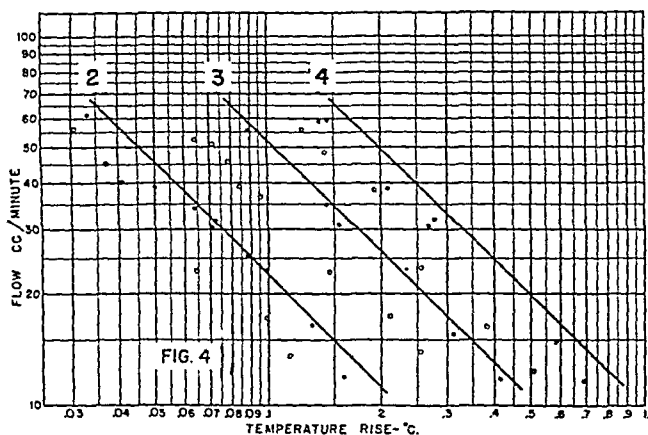


Fig. 4. Graph indicating heat transfer to water perfusing unit in terms of temperature elevation. Curves indicate theoretical 100 per cent transfer at three heating intensities. (For data see text.) Dots—thermostromuhr surrounded by air (dry cotton). Circles—thermostromuhr surrounded by water saturated cotton. 2, 3, and 4—same as in figure 1. Ordinate and abscissa—same as in figure 1.

Fig. 5. Graph showing temperature elevations of individual thermocouples of thermostromuhr with derived differential temperatures with external environments of air (dry cotton) and of muscle tissue. Heater current—200 MA. A, air environment; T, tissue environment; H, C and D, same as in figure 1. Ordinate and abscissa—same as in figure 1.

From the foregoing, it would seem likely that the thermocouples lying intimately close to the passing fluid would be particularly affected by change in environment. To investigate this point the special unit for individual couple temperature measurements was placed in a pump system and the temperatures of both couples together with the differential temperatures were determined with the exposed surface of the unit surrounded by dry cotton and then by muscle tissue at room temperature.

As seen in figure 5, the presence of tissue depresses the temperatures of both couples, but by different amounts. Since the new temperature curves do not parallel the controls, the major influence is presumably the depression of the couple temperatures directly, by affording a more rapid exit of heat from the couple areas to the external environment.

Such heat loss may become even more significant when units are applied in animals. A living tissue environment may not always be considered a *fixed* environment as with the use of dead tissue. Blood flow through the tissue may not only increase the total heat loss from the unit, but may selectively affect the area over one thermocouple more than the other (1).

*Influences, internally induced.* Additional influences affecting the heat gradients at the thermal junctions are the presence of zero flow, back flow and turbulence in the fluid passing through the unit.

In a previous paper (1) the presence of zero flow in the flow pattern caused a diminution in the galvanometer deflection, as observed in a conventional unit. To quantitate the changes in the individual couple temperatures the special unit in connection with a pump system was used. A motor operated valve permitted interruption and cessation of flow for any desired period of the cycle. Exact measurement of the flow components could be obtained from the record of an orifice plate meter (2) interposed in the line.

The effects of 35 per cent zero flow upon each of the couples separately, together with the net effect exerted upon the differential temperature curve, is presented in figure 6. These values are to be contrasted with the control curves obtained with an uninterrupted flow. The obvious change is the increase in the temperature of the cold junction.

To maintain the same flow per minute when flow ceases (zero flow) during part of each cycle, the velocity of the forward flow of the fluid through the unit must be greater than that which existed during the constant control flow. Under these conditions, the couple temperatures as recorded represent *means* derived from rapidly changing heat gradients. Since the cooling effect of the fluid is extremely small when the flow is zero and is much larger during the necessarily greater forward velocity required to give the same minute flow, the temperature of each couple would be expected to vary with the alternating components of the flow cycle. This was confirmed to the extent of observing 2 to 3 mm. oscillations of the galvanometer deflection synchronous with the interruptions in flow. The ultimate mean temperature of each couple is therefore determined by the final mean position it occupies in the rapidly changing gradients. The mean gradient about the cold junction has undergone the greater shift from the control level. The apparent indifference of the hot junction should not be regarded as indicating its invulnerability to such changes, but merely as evidence of a closer balance of the two opposing influences at that site.

That ordinary calibrations are not applicable to the measurement of flows having periods of zero flow could have been predicted on the basis of the theoretical consideration of Burton (3) and the experimental findings of Herrick, Baldes and Sedgwick (4). Studies of the operation of the Rein thermostromuhr revealed that flows of small magnitude fail to vary with the galvanometric deflection in the relation  $GV^N = K$ , in which  $G$  is the galvanometric deflection,  $V$  the flow in cubic centimeters per minute, and  $N$  and  $K$  are constants for the particular unit. Since Burton points out that the same would essentially hold

for the direct current thermostromuhr, a conventional calibration at higher flows, in which the above relation does exist, is therefore not applicable to flows in which part of each cycle has a rate of flow of a few hundredths cubic centimeter per minute, or less (near zero or zero flow). We concur in the contention that the "thermostromuhr indicates cessation of flow or very small flows by decreasing deflection" not only when such flows are prolonged, but also when either is present in the *flow cycle*.

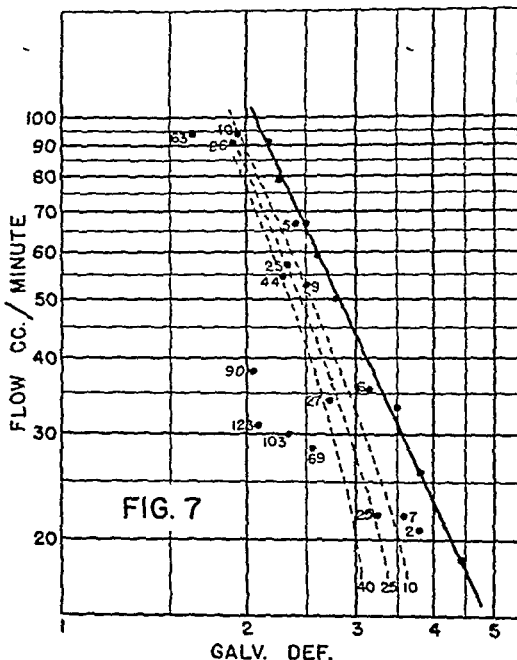
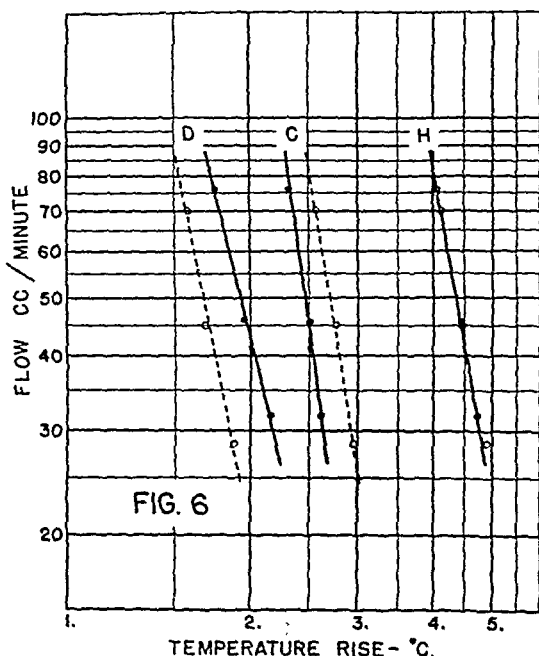


Fig. 6. Curves indicating temperature elevations of individual thermocouples of thermostromuhr with and without presence of zero flow (35 per cent of cycle time) in flow pattern of fluid metered. Heater current—400 MA. Dots—control uninterrupted flow. Circles—35 per cent zero flow in in each cycle. H, C and D—same as in figure 1. Ordinate and abscissa—same as in figure 1.

Fig. 7. Graph showing the effects of periods of back flow in the flow pattern on the galvanometric deflections obtained with a conventional thermostromuhr unit plotted against flows measured in a graduate. Continuous line—calibration with uninterrupted flow, all other conditions remaining the same. For further description see text. Ordinate—flow in cc./min. Abscissa—galvanometric deflection in 100 mm.

The effects produced by the presence of back flow in the flow pattern are not dissimilar to those discussed above. To obtain such a flow pattern the same schema was used as for the study of zero flow, except for the replacement of the stopcock with a short length of resilient rubber tubing. For the production of the back flow the tubing was partially compressed and suddenly released by a motor operated lever arm. The proportion of each cycle occupied by the period of back flow was measured from the record of the orifice plate meter, while the volume of back flow was determined by calculating the volume of "suck-back" into a piece of transparent plastic tubing attached to the peripheral end of the system.



In figure 7 are shown galvanometer readings (differential temperatures) plotted against actual flows with various volumes of back flow per cycle. The positions of these points are to be contrasted with that of the calibration curve obtained in the presence of an uninterrupted flow under the same conditions. The number near each point represents the corresponding volume back flow per cycle in cubic millimeters. Indicated by interrupted lines are the positions to which one would expect the calibration curve to shift in the presence of the volume back flow per cycle noted below each.

Back flows of a volume less than the volume of fluid existing between the heater and the cold junction (9 cu.mm. by measurement) exert a relatively small but definite effect on the calibration curve.

By use of the special unit and with the back flow volume per cycle kept as near to 44 cu.mm. as possible for the readings at three flow levels, the shift of the couple temperatures is very similar in extent to that observed in the presence of 35 per cent zero flow (fig. 6), although the interruption of the forward flow occupies only 10 per cent of the cycle or less. Small galvanometer oscillations were again observed indicating that the couple temperatures were mean values resulting from the alternation of at least two different temperature gradients.

The mechanism determining the gradients would appear to be slightly different in the case of back flow since the fluid not only ceases flowing at two points in the cycle, but the two thermocouples reverse their orientation with respect to the direction of flow for a certain part of the cycle, the "upstream" becoming "downstream" and vice versa. A consideration dealing with the interplay of these rapidly changing dynamic and static states insofar as they determine the temperature gradients around the couples would necessitate investigation beyond the scope of this paper. However, superficially a back flow of fluid carries already heated fluid past the areas from which it received part of its heat (fig. 2). The relative cooling effect upon any given area depends upon the respective temperatures of the area and the fluid going by it. The cold junction area, being at a relatively low temperature because of its originally predominate upstream position is, therefore, particularly affected by the reversal of the warmed fluid stream.

Theoretically, the thermostromuhr, when conventionally calibrated, is incapable of quantitating flow in the presence of back flow. With the Baldes and Herrick or Rein type unit the galvanometer deflection is theoretically zero when flow is zero. With equal amounts of forward flow and back flow the total flow is still zero, deflection zero and the mean flow zero. Hence, any amount of back flow will presumably nullify or neutralize to a definite extent the effects of forward flow, but the recorded empirical "mean" flow is not necessarily a difference, mean or average of the two flow components. A calibration obtained with flow proceeding in one direction only obviously cannot be applied to deflection values obtained under conditions in which the flow is periodically reversing its direction.

In the literature (5, 6, 7) and in public discussion it has been stated or im-

plied that the presence of turbulence in the fluid metered constitutes an important source of error. In anticipation of the possibility that zero flow and back flow effects could result from the coincident production of turbulent flow, a study of the production and effects of turbulence in the thermostromuhr was undertaken.

Turbulence, as defined, is a state of agitation or confusion, and as far as can be learned is incapable of quantitation. It is presumed, however, that when the conduct of a fluid as it passes through a tubular structure deviates from a "streamline" progression, such a state may then be called turbulence.

It is difficult to determine what degree of turbulence may exist in blood flowing through a blood vessel, owing to the relative opacity of the two substances. To obviate this, translucent or transparent tubes of glass, plastic, and rubber perfused with acacia solution (spec. visc. 4.0-4.5) were substituted in these studies. In the acacia solution were suspended extremely fine aluminum flakes (8) in the amount of 0.5 gm./L. Because of the small size and weight of the aluminum particles and the relatively high viscosity of the medium no appreciable settling was noted even if the mixture were left undisturbed for several minutes. With this degree of stability the conduct of the particles as observed in a microscope was assumed to reflect fairly well the conduct of the medium in the immediate vicinity.

First, attempts were made to visualize to what extent turbulence might exist under conditions similar to those in small arteries *in vivo*. A thin, translucent walled rubber tube 2 mm. in diameter was constructed so as to simulate in conformation and elasticity a small tortuous artery (fig. 8-A). The acacia mixture was pumped through this artificial artery so that the pulse waves gave rise to pulsatile expansion, flexion, torsion, and elongation of the tube. As observed through a microscope, no turbulence was noted within the tube with flows up to 100 cc. per minute. Angulation and indentation of this and other tubes of equal bore to the extent shown in figure 8-B, C, D, E, failed to induce visibly turbulent flow beyond or at the deformity.

While turbulence is not readily obtained under such conditions in a tubular structure of this size more abrupt constrictions in the bore (fig. 8-F, G) set up marked degrees of whirling turbulence in the slower moving fluid immediately beyond. (These conditions are not dissimilar to those which may exist during *in vitro* calibration of a unit.)

Having produced a definitely turbulent flow by somewhat unnatural means, the effects upon the thermostromuhr were next considered. A special unit was constructed similar to that used above, but with conventional wiring and with such precautions as to afford maximal transparency through all portions for observation under a microscope. In the upstream end of the glass tube, running through the unit, a plastic ring (similar to that shown in fig. 8-G) was placed 5 mm. from the upstream edge of the unit. During perfusion with the acacia mixture turbulence was seen to exist opposite the area of the cold junction, but no change in the galvanometer deflection from the control reading was observed. Moving the ring to positions 3, 2 and 1 mm. from the edge of

the unit permitted extension of the turbulence further into the unit resulting in the respective shifts in the deflection from the control calibration shown in figure 9. The appearance and extent of the turbulence at several flow levels has been diagrammed for one series (fig. 8-9, H, I, J, K).

Attempts to obtain similar effects under somewhat less abnormal conditions failed. A conventional unit was applied to a snugly fitting artery segment and

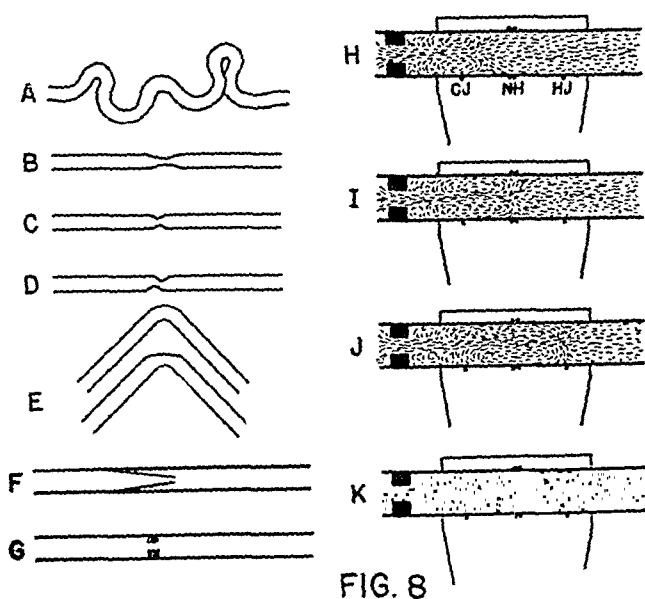


FIG. 8

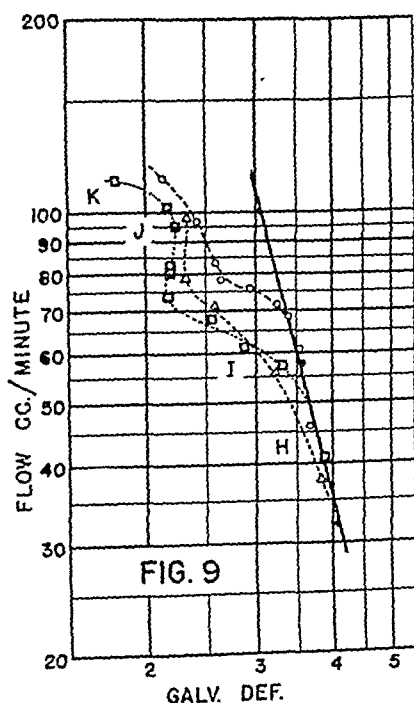


FIG. 9

Fig. 8. A, B, C, D and E, diagrams illustrating conformation and deformities of tubes through which the passage of fluid under conditions described in text gave rise to no observable turbulent flow. F, G, diagrams illustrating abrupt changes in bore of tubes by means of which decidedly turbulent flow was produced. H, I, J and K, appearance of flow showing nature of turbulence and its relation to the thermocouples and heater elements as observed through a transparent thermostromuhr unit. Letters correspond to flow levels denoted by same letters in figure 9 in relation to the curve indicated by squares. CJ, cold junction; HJ, hot junction; NH, heater.

Fig. 9. Graph showing effects of demonstrable turbulence within thermostromuhr unit upon the galvanometric deflection. Dots and uninterrupted line—calibration in the absence of turbulent flow. Circles—constricting ring placed 3 mm. from upstream edge of unit. Squares—ring placed 2 mm. away. H, I, J, and K indicate points on same curve at which turbulent patterns shown in figure 8 were observed. Triangles—ring placed 1 mm. from upstream edge of unit. Ordinate and abscissa—same as in figure 7.

a calibration obtained. Angulation of the artery at the point of entrance into the unit 90° or less failed to shift the galvanometric deflection at any flow level, provided precautions were taken to prevent variation in *artery contact* through such manipulation. Without these precautions such angulation at the downstream end alone induced marked effects, thereby dismissing turbulence *within* the unit as the causative factor.

Lastly, the ring was removed and the pump system re-arranged so as to deliver flows with either zero flow or back flow through the unit. Direct observation revealed nothing suggestive of turbulence in any part of the cycle, with wide variations in the amount of zero flow or back flow.

It is believed that the numerical data presented in this paper are applicable to the factors they delimit insofar as they indicate *qualitative* and *directional* changes. Since any quantitative data are applicable only to a given unit under given conditions, the *quantitative* significance of the data in the foregoing experiments is therefore specific only for the unit and conditions so described.

#### SUMMARY

In the application of the direct current thermostromuhr of the Baldes and Herrick type to the measurement of blood flow, several factors other than the rate of flow were found to influence the empirical flow readings, namely, 1, changes in the environment of the thermostromuhr unit, 2, the presence of zero flow, and 3, back flow in the flow pattern (1).

The mechanism of operation of the thermostromuhr was examined from the standpoint of qualitative changes produced in the heat gradients about the thermocouples. Fundamentally, the thermocouples, heated primarily by direct conduction through the block from the heater area are *cooled* by the fluid passing through the unit since their individual temperatures are found to be greater than the corresponding adjacent fluid passing by each. The extent to which each couple is thereby heated and cooled in relation to flow determines the differential temperature and therefore the galvanometer deflection at any flow. The latter relation constitutes the so-called calibration.

Changing the external environment of a unit was found to alter the thermocouple temperatures through changes in the rate of heat loss to the environment.

It is concluded that any calibration of a thermostromuhr unit is applicable to the operation of the unit only under environmental conditions identical to those under which the calibration is obtained.

This also applies to internal environmental changes where the presence of zero flow or back flow in the flow pattern of the fluid metered renders worthless the empirical mean flow values as read from a conventional calibration. Such apparent incompetence of the thermostromuhr is the direct result of an unwarranted application of an inappropriate calibration rather than evidence of an inherent physical incapability.

Turbulent flow and its influence upon the operation of the thermostromuhr were examined under several abnormal conditions. It is believed that the extent to which turbulence may naturally exist in a small, healthy blood vessel is insufficient to produce effects worth consideration in the face of more demonstrable influences which impair the accuracy of the instrument. Indeed, if turbulence of appreciable magnitude does at times occur in the arteries of normal animals, there is even less possibility of detection or control than exists in the presence of other various influential variables.

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# THE HOMEOSTATIC RÔLE OF A RENAL HUMORAL MECHANISM IN HEMORRHAGE AND SHOCK<sup>1</sup>

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Since exaggerations of normal processes often result in pathological conditions, it would be surprising if the humoral mechanism involved in renal hypertension did not have a physiological counterpart. It appeared likely that this mechanism would be evoked by various emergencies altering renal circulation, and therefore constitute an important homeostatic factor.

Wakerlin and Chobot (1939) and House and Wakerlin (1941a, 1941b) found no evidence for a specific rôle of the kidney in the regulation of normal blood pressure. In a preliminary abstract Hamilton and Collins (1941) found that the kidneys are responsible for a pressor material which aids in the maintenance of arterial blood pressure during hemorrhage. The present paper is an amplification and extension of these results. At about the same time Sapirstein, Ogden and Southard (1941) found that blood taken after hemorrhage had a renin-like action on guinea-pig ileum; the responsibility of the kidney for the development of this property was not established.

**METHODS.** Fifty-seven experiments were performed on mongrel dogs. All animals were anesthetized, usually with alpha-chloralose given intravenously as a 1 per cent solution (in Locke's fluid without glucose). The initial dose was 10 cc. per kgm. of body weight, and more was given when necessary in certain types of experiments. Combined morphine-chloralose anesthesia was used occasionally. Recipients, used as test animals, were anesthetized as above or with sodium pentobarbital. Mean arterial blood pressure was recorded from the carotid artery with a mercury manometer. All procedures involving the renal vessels, kidneys or adrenals were done through the retroperitoneal approach. Heparin was added to blood used for tests or reinjection. Blood was tested for pressor activity by observation of the changes in blood pressure resulting from injection into a small, nephrectomized dog. The blood was introduced at body temperature from a burette into the left femoral vein, while the same amount was withdrawn synchronously from the right femoral artery; changes in the blood volume of the recipient were thus avoided. Renal venous blood was obtained from the donor by means of a bent hypodermic needle attached to a syringe. Great care was taken to avoid any renal congestion or ischemia. Blood was also taken from the femoral artery and in a few cases from the inferior vena cava. The samples were tested shortly after they were taken.

**RESULTS.** 1. *Studies of the pressor activity of blood in hemorrhage.* (Table 1.) Samples of blood taken before hemorrhage from either the renal vein or

<sup>1</sup> This investigation was aided by a grant from the American Philosophical Society.

TABLE 1

*Pressor responses from the blood of animals subjected to hemorrhage*

All tests were made in nephrectomized recipients. The hemorrhages were not made at a constant rate; the time between sampling and the end of hemorrhage varied; different amounts of chloralose involving variations in fluid volume were given before hemorrhage. R, A and CV signify that the samples were taken from the renal vein, femoral artery, and inferior vena cava.

EXP. NO.	CONTROL BLD.		TOTAL BLOOD LOSS	AFTER HEMORRHAGE			
	Vol. of sample	Rise		Vol. of sample	B.P. when taken	Rise	Remarks
A. Intact animals							
	cc.	mm. Hg	cc. per kgm.	cc.	mm. Hg	mm. Hg	
1	100R	5	45	53R	50	16	B.P. of donor rising
2	46A	2	38	36R	50	24	
3	45A	3	38	40R	50	15	
4	58R	4	34	24R	60	62	Manipulative renal ischemia
5	60R	4	39	47R	100	20	B.P. of donor rising
			43	47A		9	
6	44R	0	40	32R	90	28	B.P. of donor rising
			43	32A		13	
7	42R	6	36	42R	56	18	B.P. of donor falling
			41	42A		12	
8	42R	0	34	42R	40	40	B.P. of donor falling
	42A	0	34	42A	40	40	
B. Renal denervation							
9			38	26R	53	26	B.P. of donor falling
			38	44A		22	
C. Adrenalectomized dogs							
10	23R	8	17	13R	28	10	Donor died soon after sample
11	42R	10	35	42R	60	19	B.P. of donor falling
			38	42A		14	
12	42R	Fall	25	20R	36	31	Donor died as sample was taken
13	42R	0	20	42R	50	17	B.P. of donor falling
	42A	0	25	42A		11	
14	42R	0	19	42R	54	24	
	42A	0	19	42A		20	
15	42A	2	10.9	42R	44	12	
			10.9	42A	44	9	
			16.3	42R	32	23	
			16.3	42A	32	17	
			21.3	30CV		21	Dog died as sample was taken

femoral artery of the donor gave insignificant pressor responses. The results following hemorrhage will be considered separately in the various types of experiments.

Eight intact animals were first studied (table 1, A). About one hour after hemorrhage (34–45 cc. per kgm. of body weight) bloods from the renal vein and femoral artery gave rises of 15 to 40 and 9 to 40 mm. Hg respectively. In many cases the reactions were renin-like, and persisted 10 to 30 minutes. In other cases the elevations of blood pressure lasted 3 to 10 minutes, and approximated, therefore, the duration of angiotonin responses. This difference in the duration of the responses was not related to the magnitude of the rise. In two experiments (5 and 6), where the blood pressures were relatively high at the time of sampling (100 and 90 mm. Hg), blood from the renal vein possessed a much greater activity than arterial blood. In the other experiments of this group and also in those performed on adrenalectomized dogs (table 1, C), the blood pressures were lower and the renal arterio-venous differences less. In one intact animal (expt. 8), in which the blood pressure was continuously falling, samples taken at 40 mm. Hg showed no arterio-venous difference.

In one experiment (table 1, B) with renal denervation hemorrhage resulted in good pressor responses. This finding indicates that the production of pressor material does not depend entirely on connections with the central nervous system.

The renal arterio-venous differences obtained suggest that the source of the pressor material is the kidney. The following experiments give conclusive proof for a renal origin. In six acutely adrenalectomized dogs hemorrhage uniformly resulted in the appearance of pressor material (table 1, C). In three additional experiments both kidneys and adrenals were removed, and several arterial samples were tested during the progress of the hemorrhage. Pressor material failed to appear even with lethal hemorrhage. Thus in the adrenalectomized animal the kidney is essential for pressor activity. The contribution which the adrenals may make in similar hypotension was determined in three nephrectomized animals with intact adrenals. In two cases blood from the vena cava near the orifices of the phrenico-abdominal veins gave epinephrine-like responses of 18 (expt. 20) and 75 (expt. 21) mm. Hg. The arterial sample of experiment 21 taken immediately before death gave a transitory (1 min.) epinephrine-like rise of 13 mm. Hg, and was the only arterial blood which showed pressor activity.

2. *Effect of the kidneys on the ability to maintain arterial blood pressure in hemorrhage.* If this renal humoral mechanism is important in homeostasis, the ability to maintain blood pressure in hemorrhage should be impaired in the absence of renal circulation. Observations were made on twenty-four dogs, anesthetized with chloralose. Since it was necessary to avoid inequalities in fluid administration, only the initial dose of chloralose was given.

Observations on the blood loss necessary to lower the blood pressure to 30 mm. Hg were made on eight dogs with intact renal circulation and on four nephrectomized dogs used primarily for a study of renin tachyphylaxis. Four of the animals with intact kidneys were subjected to sham-nephrectomy, consisting of bilateral renal exposure and manipulations in the wound. The hemorrhage consisted of repeated bleedings. The blood pressures of the nephrectomized



animals were lowered to 30 mm. Hg in  $1\frac{1}{8}$  hours, while in the intact animals the hemorrhage extended over 2 hours although there was a greater blood loss at a faster rate. The blood losses in cubic centimeters per kilogram of body weight were as follows: group with intact kidneys and no sham-nephrectomy—46.0, 44.2, 58.6, 45.4 (average—48.6); sham-nephrectomized group—44.2, 50.3, 45.3, 51.1 (average—47.7); nephrectomized group—42.8, 23.6, 37.6, 36.6 (average—35.2).

More carefully controlled studies were made on twelve additional dogs (4.5–11.1 kgm.). In six animals the renal vessels and ureters were tied with as little disturbance as possible to the nerves, and the kidneys were left *in situ*. Identical operations were performed in the remaining six dogs except that renal circulation was left intact and ureters were not tied. The animals were then bled; the rate and total amount of blood loss were rigidly constant (fig. 1). The falls in blood pressure occurred earlier and were greater in the dogs with interrupted renal circulation. Shortly after the last bleeding the average difference in blood pressure of the two groups was 40 mm. Hg. Half of the animals without renal circulation died shortly after completion of hemorrhage, while no fatalities occurred in the other group. Since the procedures involved minimal disturbance to the adrenals, it is unlikely that injury of these organs played a rôle in these differences.

Thus an intact renal circulation aids in the maintenance of arterial blood pressure in hemorrhage, and thereby constitutes an important compensatory mechanism.

3. *Tachyphylaxis in extreme hemorrhage.* Since repeated injections of renin result in tachyphylaxis, it appeared possible that the renal compensatory mechanism might become crippled in severe, prolonged hypotension. Experiments were therefore performed to test the hypothesis that severely bled animals should become tachyphylactic to injected renin because their kidneys had liberated large quantities of this material.

The blood pressures of ten dogs with intact renal circulation and six nephrectomized animals were lowered and then maintained by hemorrhage at shock levels (60–30 mm. Hg) for about  $1\frac{3}{4}$  hours. The pressures of the intact and of the nephrectomized dogs were maintained at about 30 mm. Hg for 0 to 40 and 30 to 65 minutes respectively. Test doses of epinephrine (0.2 cc. of 1:5000) and renin<sup>2</sup> (0.1 cc.) were given intravenously before hemorrhage and after the period of hypotension. Blood was returned when necessary to prevent death. In most cases blood or Locke's fluid was administered before the final injections, since renin was not given until the animal was capable of responding vigorously to epinephrine. After hemorrhage the responses to renin of dogs with intact renal circulation were either absent or markedly reduced, while in the nephrectomized animals the average response was slightly greater than its control. Figure 2 is a composite diagram illustrating the findings. The results of only eight of the sixteen dogs are included since sham-nephrectomies were not done in some and in others renal denervation was performed. However, the results

<sup>2</sup> We are greatly indebted to Dr. Irvine H. Page for supplying the renin.

on the eight excluded dogs coincided with the others. The difference in the response to renin after hemorrhage of dogs with and without renal circulation occurred in spite of the following conditions: 1, the average response to epinephrine was about equal in both groups; 2, pitressin gave vigorous responses in both groups; 3, the intact animals had a higher average blood pressure just before injection of renin; 4, the nephrectomized animals had been subjected to a longer and more severe hypotension.

The occurrence of increased responses to renin following the bleeding in some of the nephrectomized animals is not surprising. Wakerlin and Chobot (1939) have reported that nephrectomy increases the dog's reactivity to renin even in 1 to 3 hours. We have confirmed this observation in three dogs anesthetized with chloralose. While this potentiation complicates the interpretation of our experiments, the findings suggest that the intact animal subjected to severe

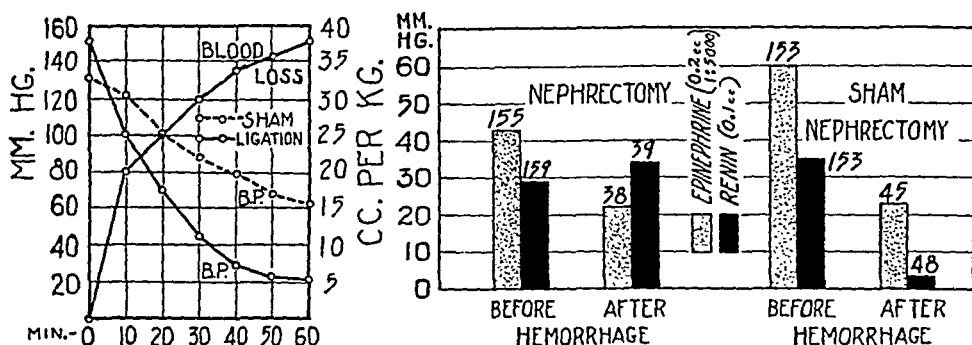


Fig. 1. Composite curves of the blood pressures of 6 dogs in which the renal vessels and ureters were tied and of 6 dogs with corresponding sham-procedures.

Fig. 2. Diagram showing the average change in blood pressure of 4 nephrectomized and 4 sham-nephrectomized dogs in response to injections of epinephrine and of renin before and after severe, prolonged hypotension produced by hemorrhage. The figure at the top of each rectangle is the average, mean blood pressure just before the injection.

hypotension for some time fails to respond well to renin because his kidneys have been liberating relatively large quantities of this material. It is interesting that under similar conditions of hypotension small renal arterio-venous differences in pressor activity were found.

Three intact animals with a less severe hemorrhage, 38 cc. per kgm. of body weight (included in fig. 1), were tested with renin before hemorrhage and one hour after its completion. In two animals the blood pressures fell to 78 and 54 mm. Hg at the end of the bleeding, and then climbed steadily. Renin was injected when the pressures were 113 and 89 mm. Hg respectively, and the responses were undiminished. Thus when the blood pressure was relatively high and compensation was occurring, tachyphylaxis was not evident. In the third dog the blood pressure fell to 54 mm. Hg, returned to 94, and then fell slowly to 78 mm. Hg. The response to renin was only 12 mm. Hg as compared to the control response of 34 mm. Hg.

4. *Histamine.* The pressor activity of the blood of three intact and three

acutely adrenalectomized dogs was studied. In order to maintain the blood pressure at shock levels (usually between 30 and 60 mm. Hg) histamine was injected in divided doses (one exception) into the external jugular vein. The intact animals required a total of 1.06 to 1.59 mgm. per kgm. body weight, while only 0.147 to 0.417 mgm. per kgm. was required in the adrenalectomized dogs. Blood removed in sampling was replaced with blood from the recipient.

TABLE 2

*Pressor responses from the blood of animals during histamine hypotension*

All tests were made in nephrectomized recipients. Repeated doses of histamine were often given to maintain hypotension. Source of sample: A = femoral artery blood, R = renal vein blood.

EXP. NO.	CONTROL BLD.		WT. OF DOG	TOTAL HISTAMINE	AFTER HISTAMINE				REMARKS
	Vol. of sample	Rise			Length of hypotension	Vol. of sample	B.P. when taken	Rise	
Intact dogs									
1	42R	0	7.7	2.6	0 : 56	42A	50	10	Bloody diarrhea Preliminary fall of 8
				7.2	1 : 40	42A	30	14	
				9.4	2 : 21	42A	46	18	
				9.4	2 : 28	42R	50	30	
2	42A	0	8.0	10.5	0 : 35	42A	34	-20	
				10.5	0 : 50	42A	56	8	
				10.5	1 : 5	42R	74	10	
				12.7	1 : 52	42A	40	14	
				12.7	2 : 10	42R	60	14	
3	42R	0	14.2	7.5	0 : 30	42A	44	12	One kidney had been removed
	42A	0		7.5	0 : 45	42R	54	22	
			15.1	1 : 30	42A	20	22		
Adrenalectomized dogs									
4	42R	0	7.2	3.0	1 : 00	42A	32	14	
	42A	0		3.0	1 : 35	42R	36	18	
5	42R	0	11.2	3.0	0 : 33	42A	26	16	
	42A	0		4.0	1 : 5	42A	36	22	
6	42A	0	7.5	4.0	1 : 25	42R	54	30	
				1.0	0 : 12	42A	44	8	
				1.1	0 : 50	42A	38	10	
				1.1	1 : 14	42R	36	30	
				1.1	2 : 23	42A	34	10	

The production of pressor material occurred in all cases, and was evident in arterial blood as early as  $\frac{1}{2}$  hour after the initial dose of histamine (table 2). In experiments where successive arterial samples were taken it was noted that the pressor activity increased with the duration of the hypotension. Although the hypotension was equivalent in degree and length to that of severely bled animals, large renal arterio-venous differences were found in about half of these

experiments. A fall of blood pressure, presumably due to histamine, was obtained from two early blood samples of a dog with a bloody diarrhea (expt. 2). Otherwise the presence of histamine was not detected in recipients even, in one case, as early as eight minutes after a histamine injection of 2.3 mgm. into the donor. Finally the renal source of the pressor material was established in two of the adrenalectomized animals; removal of their kidneys resulted in the disappearance of pressor activity from arterial blood.

5. *Adrenalectomy.* In the present investigation fifteen dogs were adrenalectomized primarily to eliminate the liberation of epinephrine. However, during the 4 or 5 hours following adrenalectomy additional interesting and surprising findings were observed which warrant reporting.

Since the removal of both adrenals at one operation often causes "shock," special care was taken to select dogs in excellent condition. After operation the dogs were left undisturbed for about 2 hours before control renal and femoral samples were taken. Control bloods of three of these animals caused marked falls in the blood pressure of the recipients. Donor 1 had a pressure of 86 mm. Hg. after withdrawal of the samples. When the renal sample was injected into a nephrectomized recipient, the blood pressure fell 130 mm. Hg, and remained low for a long time. However, the femoral sample produced no depression after standing at 37°C. for  $1\frac{1}{6}$  hours, but the donor's blood still showed depressor activity (fall of 39 mm. Hg)  $3\frac{2}{3}$  hours after adrenalectomy. Donor 2 had a blood pressure of 94 mm. Hg after withdrawal of control samples. The recipient's pressure fell 67 mm. Hg after injection of the renal sample, and remained depressed. A later sample, taken  $2\frac{5}{12}$  hours after adrenalectomy, showed no depressor activity. When a total of 25 cc. of blood per kgm. of body weight had been removed from this donor, a 20 cc. sample of renal vein blood, obtained  $4\frac{1}{3}$  hours after adrenalectomy, produced a rise of 31 mm. Hg in the recipient's blood pressure. Withdrawal of this sample caused death of the animal. A control arterial sample of donor 3 produced a fall of 38 mm. Hg in the recipient, and another sample taken  $2\frac{5}{8}$  hours after operation still showed depressor activity. Cross agglutination tests were made in this experiment, and the bloods were compatible. It is interesting that small amounts of blood (42 cc.) from donors whose blood pressures were above shock levels gave extreme falls in nephrectomized recipients.

On the assumption that the liberation of depressor material resulted from the surgical technique, the remaining adrenalectomies were performed under deeper anesthesia, the phrenico-abdominal veins were first ligated, and the other vessels, nerves, and tissues were severed by electro-coagulation instead of by ligation and cutting. The blood of ten dogs adrenalectomized by this improved technique showed no depressor activity. In spite of blood pressures within the normal range, adrenalectomized dogs were very sensitive to hemorrhage and to histamine. The withdrawal of 10 cc. of blood per kgm. gave sharp falls in blood pressure with little tendency toward recovery, while most intact animals showed little change after the loss of 20 cc. per kgm. at one bleeding. Regardless of the degree of hemorrhage, when the blood pressures of the adrenalectomized

animals were reduced to a low level, the renal arterio-venous differences in pressor material were small. Finally, adrenalectomized dogs, as compared with intact animals, required only about  $\frac{1}{5}$  as much histamine to maintain their blood pressures between 30 and 60 mm. Hg. Since the histamine was given  $2\frac{1}{4}$  to  $2\frac{1}{2}$  hours after the adrenalectomy, this marked sensitivity developed within this period.

**DISCUSSION.** Our experiments demonstrate that the kidney is responsible for the development of pressor activity in blood both after hemorrhage and after histamine injection. These findings and the report by Taquini (1938) that vasoconstrictor properties appear in renal vein blood after asphyxia suggest that the renal humoral mechanism may be involved in a wide variety of emergencies. The kidney is thus an endocrine gland, and its internal secretion is liberated in various types of stress. That the kidney aids in homeostasis is shown by the observation that absence of renal circulation impairs the ability to maintain blood pressure in hemorrhage.

We have made no consistent attempt to determine threshold values for time or degree of hypotension necessary to evoke the renal mechanism. Pressor activity was noted in arterial blood after  $\frac{1}{2}$  hour of hypotension, but considerable pressor material might have been produced before it could be detected in arterial blood by our method. The experiments concerning the effect of an intact renal circulation on the ability to maintain blood pressure suggest that the renal mechanism may operate after a relatively mild renal stimulus. Animals without renal circulation showed a marked drop of blood pressure shortly after the initial bleeding of 20 cc. per kgm. of body weight, while the fall was relatively small in intact dogs. This early involvement of the renal humoral mechanism is consistent with data from studies on renal hypertension. Verney and Vogt (1938), Braun-Menendez and Fasciolo (1939), Friedman, Seltzer and Sampson (1940-41) and others have reported that only a short period of partial constriction of the renal artery (in some instances a few minutes) is necessary before the blood pressure of the animal rises or vasoconstrictor material can be detected in renal vein blood.

Our findings on tachyphylaxis and the observation that blood after hemorrhage has a renin-like action on ileum (Sapirstein, Ogden and Southard, 1941) suggest but do not prove that the humoral mechanism involved in our experiments is the renin-angiotonin system. The nature of this system renders it susceptible to factors affecting either the liberation of renin or the availability of renin-activator. The liberation of renin would be determined by the degree and duration of the change in renal circulation. Both epinephrine and the renal vasomotor nerves are capable of affecting renal circulation, and may thus influence renin liberation. While adrenalectomy or renal denervation did not prevent the eventual development of pressor activity as a result of hypotension, we believe that these procedures may have a quantitative effect.

Continuous infusions of appropriate amounts of renin produce an excess of renin in the blood and a diminution of renin-activator, followed by tachyphylaxis and disappearance of the initial pressor effect (Page, 1939). In our experi-

ments on hemorrhage, tests in nephrectomized recipients demonstrated the continuous presence of renin-like material in the blood of donor animals subjected to severe, prolonged hypotension; animals under this condition developed tachyphylaxis to injected renin. Exhaustion of renin-activator would be accelerated by the loss of blood containing this substance. Furthermore, the pressor activity of arterial blood approached that of renal vein blood. However, tachyphylaxis was not evident and large renal arterio-venous differences were observed when the blood pressure was well above the critical level after hemorrhage. Under this condition the renin-angiotonin system is presumably operating, since large renal arterio-venous differences and absence of tachyphylaxis were observed by Page (1940, 1941) in experimental renal hypertension. In the histamine experiments, large renal arterio-venous differences were still present in about half of the animals after an hour or more of similar hypotension. This finding may be explained by smaller loss of renin-activator; there was no blood loss, and blood removed in sampling was replaced by blood rich in activator (i.e., from a nephrectomized animal).

#### SUMMARY

The pressor activity of arterial and renal vein blood was studied by determining the change in blood pressure resulting from injection into a nephrectomized recipient. Control samples did not possess significant pressor activity. After hemorrhage pressor properties consistently appeared in blood from normal dogs, from adrenalectomized dogs, and from one animal with denervated kidneys. The pressor responses in the recipient were often renin-like, persisting 10 to 30 minutes. Other reactions of equal magnitude were shorter, their duration approaching that of responses to angiotonin. Similar observations were made on dogs injected with sufficient histamine to lower their blood pressures to shock levels. That the kidney was responsible for the development of pressor activity was indicated by two findings: 1, renal vein blood usually gave greater responses than the corresponding arterial blood; 2, pressor reactions were obtained from the blood of adrenalectomized dogs, but not from dogs with both kidneys and adrenals removed.

The absence of renal circulation impaired the ability of dogs to maintain arterial blood pressure in hemorrhage.

The above findings indicate that either hemorrhage or histamine evokes a humoral mechanism in the kidney and that this mechanism aids in the maintenance of arterial blood pressure.

Intact animals whose blood pressures had been maintained by hemorrhage at shock levels for about  $1\frac{3}{4}$  hours reacted poorly or not at all to injected renin. A group of nephrectomized dogs under similar conditions gave an average response to renin which was slightly greater than the response before hemorrhage. These findings suggest that the kidneys may liberate enough renin to cause tachyphylaxis and thus disable their own compensatory mechanism. Under similar circumstances responses from arterial blood closely approached those from renal vein blood. With less severe hypotension and evident com-

compensation, tachyphylaxis to renin was not observed, and large renal arterio-venous differences were present.

Marked falls of blood pressure were produced in acutely adrenalectomized dogs by relatively mild hemorrhage or small histamine dosage. This increased sensitivity was evident  $2\frac{1}{4}$  hours following adrenalectomy.

Falls of blood pressure to shock levels occurred in the recipients upon injection of control blood of 3 dogs, acutely adrenalectomized by ligation and cutting of the nerves and vessels instead of by electro-coagulation.

We wish to express our appreciation for the valuable technical assistance of A. Sokalchuk.

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# THE EFFECT OF A CREAM MEAL ON THE ACIDITY AND NEUTRALIZING ABILITY OF THE CONTENTS OF THE DUODENAL BULB IN NORMAL DOGS<sup>1</sup>

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The inhibitory effect of fat on gastric secretion and motility has been well established and the mechanism of this action has been the subject of numerous investigations (9). Little attention, however, has been given to the effect of fat on the reaction in the duodenum (7, 8, 11), especially as regards the acidity changes in this area during the period of active digestion of the fat. Even less is known of the changes in the reaction and neutralizing ability of the contents of the duodenal bulb following the use of fat. The latter is of some practical importance inasmuch as fats have been advocated as useful agents in the management of patients with duodenal ulcer primarily because of their gastric inhibitory action.

This study was undertaken for the purpose of supplying some knowledge of the effects of fat on the acidity and neutralizing ability of the contents of the first part of the duodenum. It was hoped that by comparison with the action of other foodstuffs studied in a similar manner (2, 4) some conclusions could be drawn concerning the influence of the type of food undergoing digestion on the acidity of the duodenal contents. It was further hoped that such a comparison would help to determine whether or not there is a close correlation between the acidity of the gastric and the duodenal contents.

**METHOD.** Dogs, prepared with cannulated gastric and duodenal fistulas (10, 13), were trained and maintained as described previously (2). As in the previous study, pH and free and total acidity were measured on fractional samples removed simultaneously from the pyloric antrum and the duodenal bulb at 10 minute intervals for  $\frac{1}{2}$  hour in the fasting state and for  $2\frac{1}{2}$  hours after the beginning of the meal and at  $\frac{1}{2}$  hour intervals for an additional hour. The excess neutralizing ability of the duodenal contents was likewise estimated as before.

As a representative fat regular table cream (20 per cent fat) was selected. This was given in 500 cc. quantities so as to duplicate the amount of beef extract solution utilized in another but similar study (4). The cream was fed to all the dogs with the exception of one dog who refused to drink the entire amount, requiring introduction of the remainder through the gastric instillation tube.

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Twenty-three experiments were performed on 5 dogs. In all 2886 different observations were made consisting of 874 determinations of pH, 804 measurements of free acid, 803 measurements of total acidity and 405 measurements of the excess neutralizing ability of the duodenal contents. In expressing the results the 4 specimens collected in the fasting state were averaged to obtain a single fasting value.

**RESULTS. Acidity in pH units.** (Fig. 1.) The average acidity as expressed in terms of pH both in the stomach and in the duodenum was decidedly less than that following a carbohydrate (2) or a protein-histamine meal (4). Not only was the average gastric pH higher but the range of pH values was wider than that encountered after the other meals.

In the duodenal bulb especially the decrease in hydrogen ion concentration was very striking. The average post-meal pH value here was roughly a full unit greater than that after the meat extract-histamine meal and  $\frac{3}{4}$  of a unit greater than the average pH following the Ewald meal. The comparative depression of duodenal acidity is further emphasized by the fact that only 1.1 per cent of all the post-cream meal specimens were positive for free acid (pH 3.5 or less) (3), whereas 16.4 per cent of all the duodenal post-Ewald meal samples and 26.5 per cent of all those following the Liebig's extract-histamine stimulation had a pH of 3.5 or less.

Lack of correlation between gastric and duodenal acidity is again seen in the more or less stabilized pH in the duodenum at the same time that the stomach contents display a rising hydrogen ion concentration.

The total duration of the post-meal observation period was  $3\frac{1}{2}$  hours. Our failure to note any secondary stimulation of gastric secretory activity such as described by Babkin (1) after the feeding of fat along with other foods is perhaps due to the fact that our observations were not continued long enough.

**Free acid.** (Fig. 2.) As was to be expected, the gastric free acid values were decidedly lower than following other types of meals studied previously (2, 4). Not only was the average value less but the range of all the readings obtained was only half that seen with bread and water or meat extract-histamine stimulation. Illustrative of the previously noted lack of uniform correlation between the several indexes of gastric acidity (2, 4) is the relatively stable value for average free acid as compared with the steady increase in the average hydrogen ion concentration.

The free acid values of the contents of the first part of the duodenum afford arresting evidence of the marked contribution which cream makes to the neutralizing power of the contents of this region. Although electrometric pH measurements showed the presence of free acid in a small percentage of the samples, the indicator method failed to reveal free acid in any of the post-meal duodenal samples (3). This is in contrast with the demonstration by the same method of free acid in 2.6 per cent of the post-Ewald and 13.1 per cent of the post-meat extract-histamine specimens respectively. It must be admitted, however, that the recognition of the presence of free acid through the use of Toepfer's reagent in duodenal samples containing cream is difficult and probably

erroneous readings were made in some instances even though we attempted to remove the fat by centrifugalization.

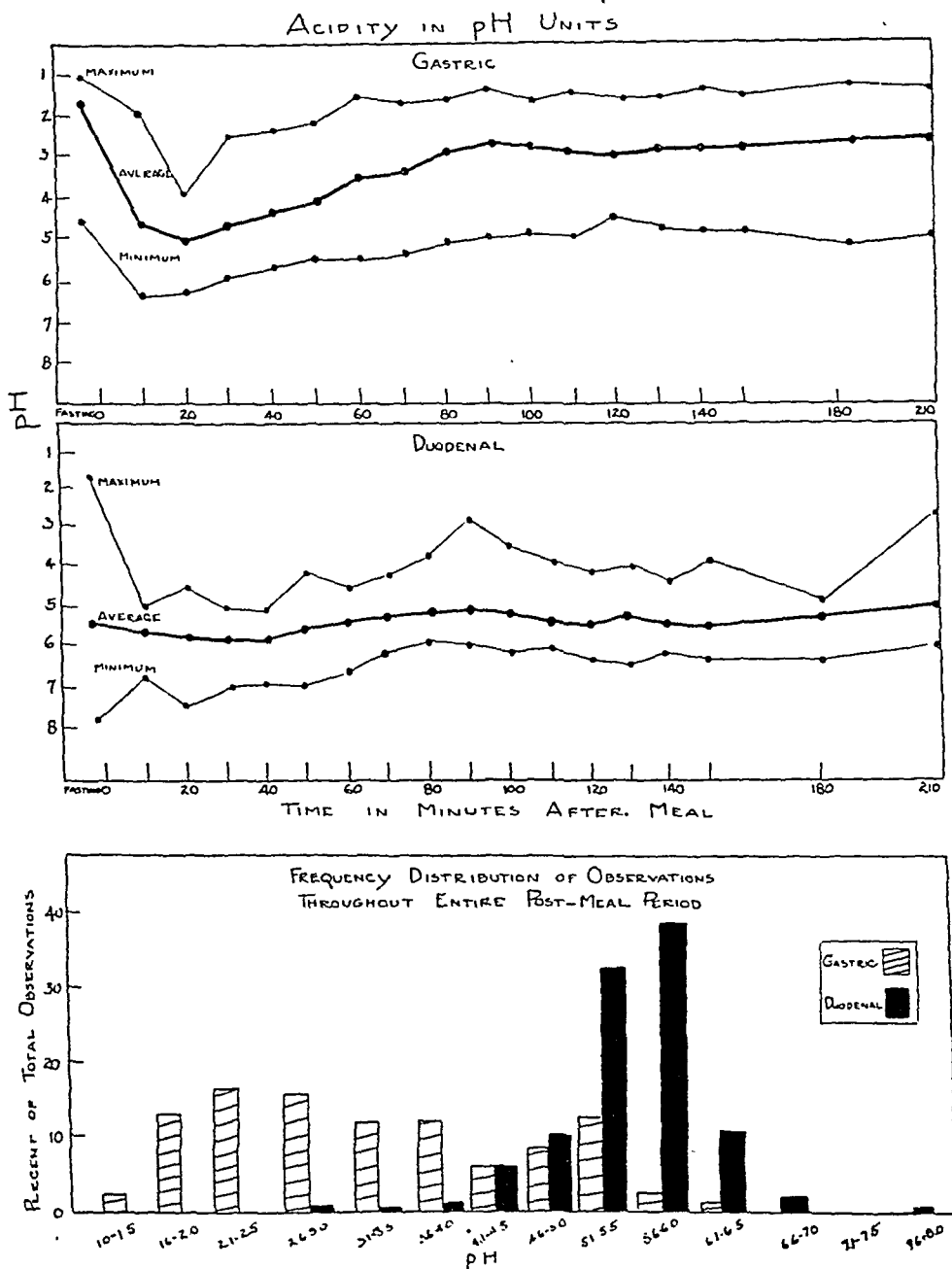


Fig. 1. Acidity in pH units of samples collected simultaneously from just above and just below the pylorus.

It might be well to point out what others have previously remarked on (5), namely, the absence of free acid does not bespeak neutralization in the chemical sense. This is illustrated by the fact that the average pH of the duodenal con-

tents never exceeded the neutral point of 7.0 despite the persistent anacidity as measured by Toepfer's reagent.

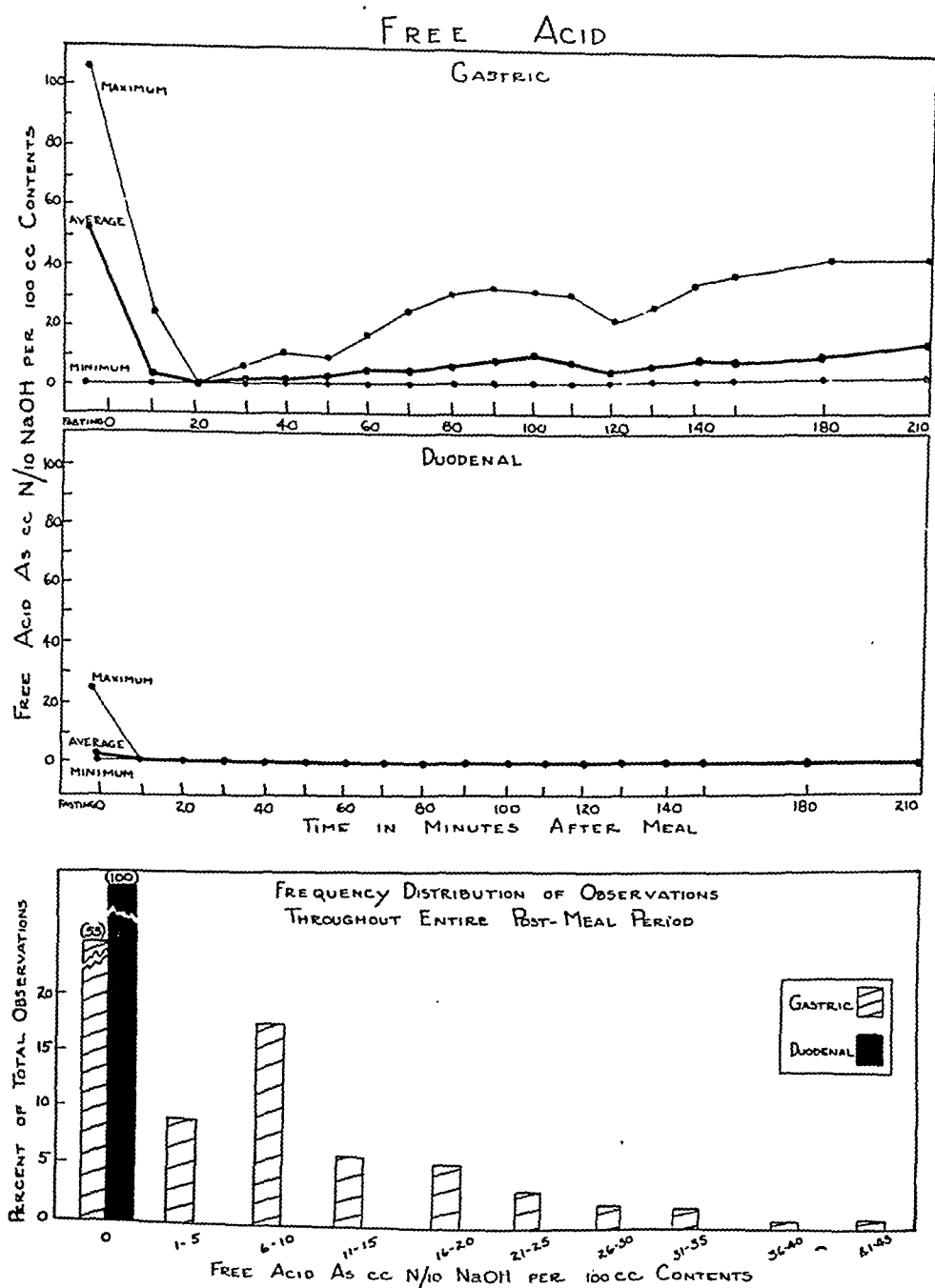


Fig. 2. Free acid as determined on samples collected simultaneously from just above and just below the pylorus.

*Total acidity.* (Fig. 3.) Again as might be expected the average total titratable acidity of the gastric contents is less following the exhibition of fat than after the use of the other foodstuffs. In the first part of the duodenum, how-

ever, in spite of the higher pH values the average total acidity as found by titration is about equal to that found after meat extract-histamine stimulation

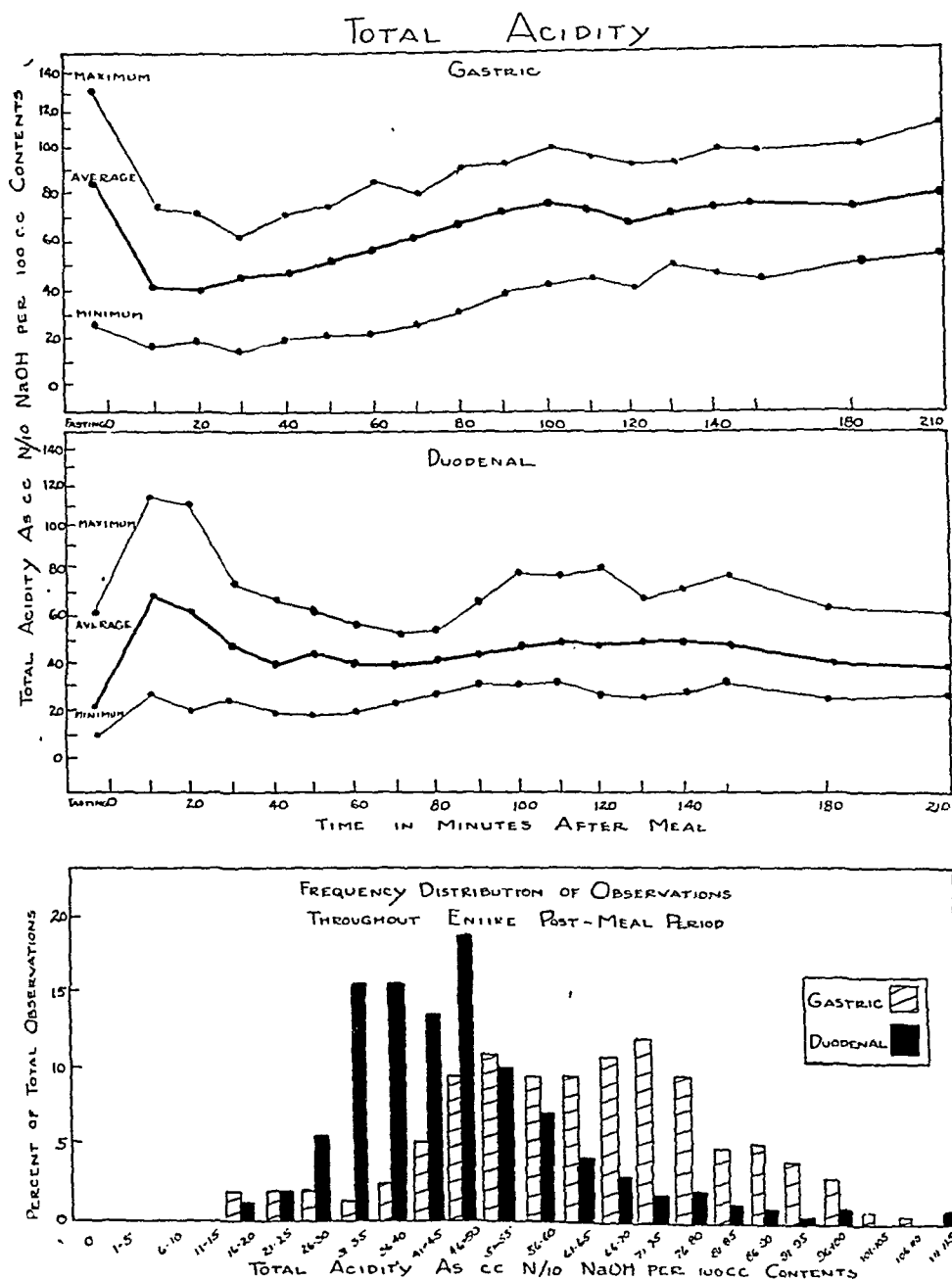


Fig. 3. Total acidity of samples collected simultaneously from just above and just below the pylorus.

and is greater than that subsequent to carbohydrate-water stimulation. Most of the values, furthermore, cluster about a higher range than do those with either of the other stimuli. This may represent the effect of the buffer action of the

soaps contained in the duodenal contents as a result of the interaction between the fatty acids and the alkaline salts of the secretions.

The curve obtained by plotting the average values of duodenal total acidity simulates slightly, if at all, that seen in the stomach and in the latter part of the post-meal period diverges from it completely. This relationship emphasizes further the lack of constant or close correlation between the acidity simultaneously determined in the pyloric and the duodenal contents.

A peculiar phenomenon whose explanation is not clear is the initial rise in

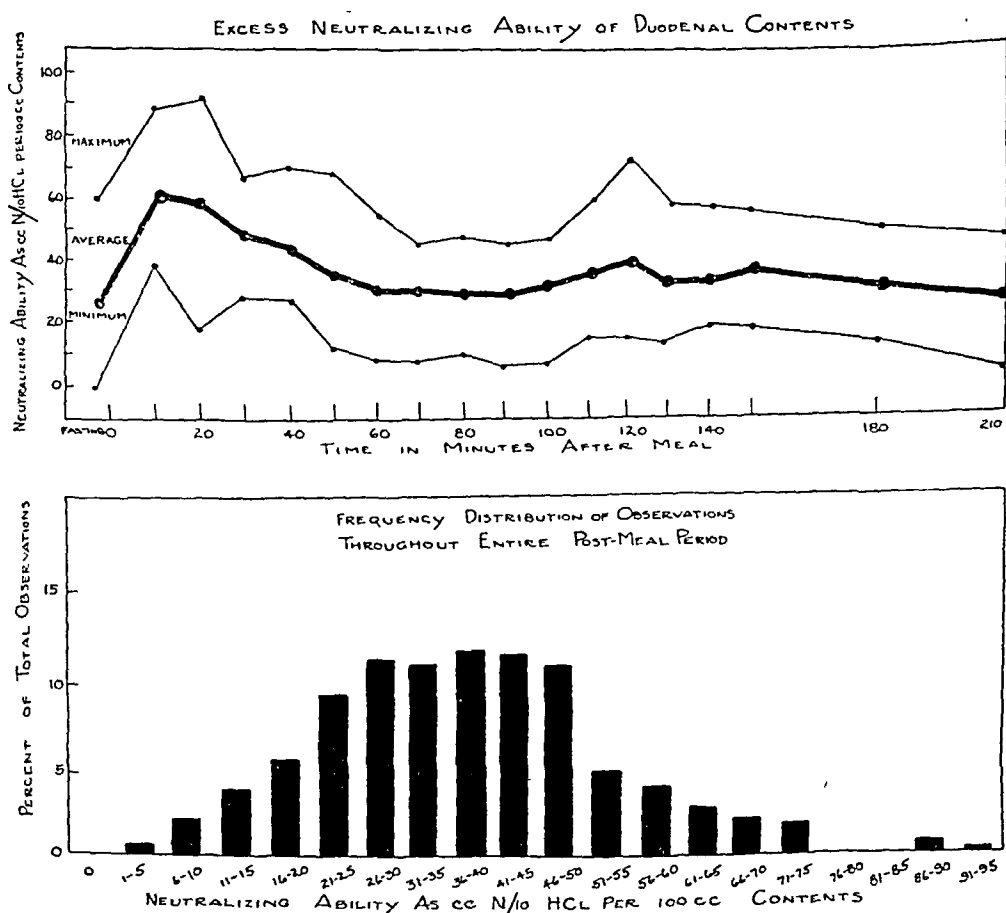


Fig. 4. Excess neutralizing ability of the contents of the first part of the duodenum.

total acidity in the duodenum in the first 20 minutes postprandial as compared with the initial fall in total acidity in the stomach during the same period.

*Excess neutralizing ability of the duodenal contents.* (Fig. 4.) As defined and described previously (2, 3), the excess neutralizing ability of the contents of the duodenal bulb is a measure of the reserve capacity which these contents possess to neutralize, buffer and dilute the chyme received from the stomach above that necessary to offset the free acid content. It was estimated by adding N/100 hydrochloric acid to a 1 cc. sample of the strained, admixed duodenal contents containing Toepfer's reagent until the color end point used for the titration of free acid was reached.

The average excess neutralizing ability of the duodenal contents was a little greater than that after the Ewald and beef extract-histamine meals; the range of the values was as great as with the bread and water stimulus and most of the values clustered about a higher range than those following the other meals. Moreover, none of the specimens failed (colorimetrically) to show some excess neutralizing capacity, whereas 2.7 per cent of the post-Ewald and 13.8 per cent of the post-Liebig's extract-histamine meal samples displayed an absence of this reserve capacity.

A remarkable finding was the abrupt initial rise in the average value of the excess neutralizing ability during the first 20 minutes postcibal. This observation coupled with the initial rise in duodenal total acidity without any marked change in the duodenal pH during the same period suggests the presence of an excess of efficient buffer in the duodenal contents at the time.

**DISCUSSION.** The inhibitory effect of fat on gastric secretion has been long appreciated and amply verified so that the observations made in this study merely lend added confirmation of that action.

One of us (11) previously provided evidence that fat (olive oil or beef fat) modifies gastro-intestinal activity in such a manner as to render the duodenal contents neutral or only slightly acid as determined by the pH of the contents removed from a point about 6 to 8 inches beyond the pylorus. Other investigators have likewise demonstrated a similar decrease in hydrogen ion concentration in approximately the same portions of the duodenum following the feeding of fat in dogs (7) and in man (8). An enhanced neutralizing capacity of the duodenal contents in the same area has also been shown after a fat meal (7). It is of great interest to us, therefore, that this study demonstrates a similar diminution of acidity and an increase in neutralizing ability of the contents of the first part of the duodenum—an area of even greater clinical interest from the standpoint of peptic ulcer.

It has been assumed that the inhibitory influence of fat on gastric secretion is probably mainly responsible for its effect on duodenal pH (7, 11). The lack of any close correlation between the gastric and duodenal pH or total acidity as well as the apparent independence of the duodenal pH and the titratable gastric acidity has been emphasized in the description of our results. Such findings suggest that the degree of gastric acidity is not quite so dominant a factor in determining the duodenal reaction as it has been considered. The effective acidity (hydrogen ion concentration) of the contents of the duodenal bulb following a cream meal is probably a resultant of the interaction of several factors chief among which are 1, the amount of acid entering from the stomach; 2, the degree of pancreatic response (8), especially of bicarbonate; 3, the buffer effect of the soaps formed in the duodenum; 4, the amount of weak, fatty acids contained; 5, and the diluent effect of the liquid meal.

Cream exerted a greater depressant effect on duodenal acidity both as regards the average values and the distribution curves than did meat (12), carbohydrate (2) or Liebig's extract and histamine (4). Moreover, it appears that cream has even a greater effect on duodenal than on gastric acidity. These findings coupled with the lack of sharp relationship between the gastric and

duodenal acidities lend support to the conclusion that the acidity of the duodenal contents in the normal dog is largely determined by the type of food undergoing digestion and is related in part only to the acidity of the gastric contents.

The effect of some of the commonly used antacids on the acidity of the contents of the duodenal bulb in duodenal ulcer patients has been studied by us and will be subsequently reported. Their action for the most part proved to be short and often negligible. The much more efficient action of cream in reducing duodenal acidity over a prolonged period in dogs suggests the preferential use of this foodstuff when a duodenal antacid effect is desired. It is quite true that the amount of cream used in these experiments is much greater than that which would be employed in therapy in patients. The small doses which would be required might prove to be ineffective, yet there is evidence that small doses of fat (olive or cod-liver oil) depress gastric pouch secretion effectively and over long periods (6) so that one might expect a similar effective action in the duodenum from small doses of cream.

#### SUMMARY AND CONCLUSIONS

1. Fat in the form of table cream (20 per cent fat) effectively diminishes the acidity and enhances the excess neutralizing ability of the contents of the first part of the duodenum over fairly prolonged periods of time in the normal dog.

2. The decrease in duodenal acidity following cream is only partly due to the inhibitory effect of the fat on gastric secretion.

3. There is no parallel relationship between the effective acidity (hydrogen ion concentration) of the duodenal contents and any of the customary measures of gastric acidity in the normal dog.

4. The acidity of the contents of the duodenal bulb in the normal dog is largely determined by the type of food undergoing digestion and is related in part only to the acidity of the gastric contents.

5. Cream appears to be a much more effective duodenal "antacid" than some of the commonly employed antacid medications.

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# PRECOCIOUS GONADAL DEVELOPMENT OCCURRING IN IMMATURE RATS FOLLOWING A SHORT-TIME TREATMENT WITH ANTIGONADOTROPIC SERUM<sup>1</sup>

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The administration of gonadotropic extracts to immature rats results in an increase in weight of the gonads that is only temporary in nature and is not accompanied by normal cyclic changes in the vaginal smears. It is conceivable, however, that a premature stimulation of the ovaries with continuous normal cyclic fluctuations in the vagina might follow gonadotropic hypersecretion of the animal's hypophysis. In preliminary reports (1) we have shown that an increase in the number of basophiles in the pituitary gland of the rat occurs during antigonadotropic treatment. After cessation of treatment there is a hypersecretion of the gonadotropic hormone accompanied by a decrease in the number of basophiles (1, 2). Severinghaus and Thompson (3, 4) have also reported basophilism in the pituitary gland of animals repeatedly injected with either sheep pituitary extracts or antigonadotropic sera. Inasmuch as an increased secretion of the pituitary gonadotropic complex follows antihormone treatment it became of interest to ascertain whether premature development and function of the gonads would follow in immature female rats after administration of antigonadotropic serum. This report extends the preliminary observations already made (1, 2) on the effect of antigonadotropic substances upon the physiology and histology of the pituitary gland.

**PROCEDURE AND RESULTS.** The source of the antihormone used in these experiments was an aspecific gonadotropic antiserum obtained from the blood of rabbits repeatedly injected with an aqueous supercentrifuged extract of sheep pituitary glands. The blood was removed by cardiac puncture and the serum prepared by centrifugation. The inhibitory action of the serum was determined by injecting the serum together with each of the following gonadotropic preparations: extracts of whole sheep pituitary glands, pregnancy urine and pregnant-mare serum and aqueous suspensions of dried human and rat hypophyses. In addition to preventing the gonad stimulating action of these extracts the antiserum also inhibited the endogenous gonadotropic secretion of rat hypophyses (5).

Immature female rats were injected subcutaneously with 0.5 cc. of the antigonadotropic rabbit serum per day—or a total dose of 5 cc.—from the 10th to the 19th day of life, inclusive, to determine the effect of gonadotropic antisera upon the pituitary gonadotropic secretion. Nine of these animals were killed on the 20th day. The average ovarian weight of the injected animals was

<sup>1</sup> Aided in part by a grant from the Wisconsin Alumni Research Foundation and by assistance given by the personnel of the W.P.A. Official Project no. 65-1-53-2349.



5.3 mgm. as compared to 8.9 for 8 untreated littermate control animals killed at the same time. Sections of the gonads of the animals given antihormone did not show follicles with antra which are found in the ovaries of control animals of the same age. The condition of the ovary in these animals indicates that the gonad stimulating hormone of the pituitary gland was prevented from producing its characteristic effect.

The pituitary glands of the antihormone treated and control animals were removed at autopsy and fixed in Bouin's fluid. They were sectioned at 6 microns and stained with Rasmussen's modification of Mallory's trichrome stain (6).

TABLE 1

*Increase in ovarian weight of immature rats following cessation of antigonadotropic treatment*

TREATMENT	NO. OF RATS	DAY KILLED	OVARIAN WEIGHT	UTERINE WEIGHT
		<i>age</i>	<i>mgm.</i>	<i>mgm.</i>
Experimental*.....	9	20	5.3	12.4
Control.....	8	20	8.9	19
Experimental.....	7	22	7.8	17
Control.....	7	22	10.6	19
Experimental.....	7	24	15	39
Control.....	5	24	11	20
Experimental.....	7	26	21	87
Control.....	6	26	12	31
Experimental.....	7	28	27	119
Control.....	4	28	13	26.5
Experimental.....	8	31	48	
Control.....	8	31	15	
Experimental.....	7	34	54	148
Control.....	5	34	16	29

\* Injected with sheep antigonadotropic rabbit serum from the 10th to the 19th day of life, inclusive.

The pituitary glands of the rats treated with the antigonadotropic serum exhibited a picture of extreme basophilism when compared with those of littermate controls. The degree of basophilism found in the injected rats was the same as that found in castrated rats. The results of a quantitative study of the changes in the three cell types of the pituitary gland have been presented in an earlier paper (1) where it was reported that after a 10 day period of antihormone injections 31.9 per cent of all the cells were basophiles, in contrast to 5 to 10 per cent found in the littermate controls. A pituitary gland showing such a degree of basophilism could be expected to exhibit an increase in gonadotropic secretion (7). Hence upon withdrawal of the antiserum further neutralization of the endogenous

gonadotropic hormone by the antiserum would be prevented and the gonad stimulating hormone secreted by the basophiles could act on the gonads. With this thought in mind, rats treated with 0.5 cc. of antigonadotropic serum per day from the 10th to the 19th day of life, inclusive, and uninjected littermate controls were killed 1, 3, 5, 7, 9, 12 and 15 days after cessation of the 10 day treatment with antiserum. The data in table 1 show that the ovaries and the uteri of the animals killed at successive intervals after antihormone treatment exhibited a progressive increase in weight from a subnormal value found immediately after the injections were discontinued, to one much greater than that found in control animals. Accompanying the increase in weight and activity of the ovaries of the antihormone treated animals there was a progressive decrease in basophilic cells until the 15th day after the injections were discontinued when the normal picture again prevailed. The reduction in number of basophiles may be the result of the ovarian secretions produced after the antihormone treatment was stopped. This theory is supported by results obtained in an experiment in which all the animals in three litters of six female rats each were treated with 0.5 cc. of antigonadotropic serum per day from the 10th to the 19th day of life inclusive. One third of these animals in each litter was killed on the 20th day of life; another third was castrated on the 20th day and was killed with the remaining rats on the 34th day of life, or 15 days after cessation of injections. The pituitary glands of the antihormone treated animals which were castrated immediately following the last injection exhibited approximately the same degree of basophilism noted at the time that the injections were stopped. A normal picture was found in the pituitary glands of littermate females that were not castrated after cessation of antihormone treatment, but were killed on the 34th day of life. The average ovarian weight of these animals had increased to 63 mgm. as compared to the control ovarian weight of 16 mgm. The enlargement of the ovaries was undoubtedly accompanied by an increase in the amount of gonadal hormones which might be the cause of the concomitant decrease in the number of basophiles.

Forty-two additional female rats were treated from the 10th to the 19th day of life, inclusive, with 0.5 cc. of antigonadotropic serum per day to obtain further evidence concerning the induced physiological activity of the pituitary gland of the antihormone treated animal as determined by the effects on the gonads and vaginal smears. The time of vaginal opening after cessation of antigonadotropic injections on the 20th day was noted for all the experimental females. Twenty-four of these animals were killed 5 days after opening of the vagina to determine the extent of ovarian hypertrophy present at that time. Similar observations were made of littermate control rats of the same age. The data in table 2 show that the average age of rupture of the vaginal membrane of the 24 antihormone treated animals was 28.5 days and the ovarian weight 5 days later was 42 mgm. Eighteen littermate control rats killed at the same time had an average ovarian weight of 16.1 mgm. The ovaries of the experimental rats showed large cystic follicles, corpora lutea and blood points, whereas those of the control animals were juvenile in appearance. Since the control animals were

killed prior to the time when opening of their vaginas would have normally occurred, the time of opening in the experimental rats treated with antihormone could not be compared with that of littermates. It was necessary, therefore, to make the comparison with non-littermate rats injected with normal rabbit serum. As the average age of vaginal rupture of these animals was 48 days (table 2) it is apparent that the sexual development of the antihormone pretreated animals was accelerated 19 days before its normal occurrence.

The 18 remaining rats that were treated from the 10th to the 19th day of life, inclusive, with antihormone were not autopsied until at least 70 days of age. Daily vaginal smears were made from the time that the vagina opened until

TABLE 2  
*Response of the immature rat to pretreatment with antigonadotropic sera*

TREATMENT	NO. OF RATS	TOTAL DOSE	OVARIAN WEIGHT	UTERINE WEIGHT	AGE AT O. V.†
		cc.	mgm.	mgm.	
AntiSAP* 10th to the 19th day of life, inclusive, and killed 5 days after O.V.†.....	24	5	42		28.5
Littermate controls.....	18		16.1		Not open
AntiSAP 1st to the 10th day of life, inclusive, and killed 5 days after O.V.....	13	1	17	124‡	21.5
Littermate controls.....	12		15.5	25.6‡	Not open
Normal rabbit serum 10th to the 19th day of life, inclusive, and killed at 41 days of age.....	11	5	17		Not open
Littermate controls.....	7		17		Not open
Normal rabbit serum 10th to the 19th day of life, inclusive.....	14	5			48.2
Littermate controls.....	11				47

\* Sheep antigonadotropic rabbit serum.

† Opening of the vagina.

‡ Three rats.

autopsy. Littermate control rats were likewise observed and both the experimental and control animals were killed at the same age after recording at least three successive estrous periods for the control animals. The vaginal smears showed that the experimental animals experienced at least one or two successive estrous periods prior to the time of vaginal opening in the control females. The experimental and control ovarian weights obtained at autopsy were essentially the same, indicating that the hypersecretion of the pituitary gland found soon after stopping the antihormone treatment was only transitory.

In view of these results it became of interest to determine whether the premature opening of the vagina with the accompanying ovarian development and cyclic variations in the vaginal smear could be made to occur earlier by beginning the antigonadotropic treatment of the rats on the first day of life and continuing

it for ten days. With this idea in mind, 35 female rats were injected subcutaneously from the 1st to the 10th day of life, inclusive, with 0.1 cc. per day or a total dose of 1 cc. of antiserum. The time of vaginal opening after cessation of antihormone injection was determined and thirteen animals of this group were killed five days after their vaginas had opened. The results presented in table 2 show that pretreatment with antigonadotropic serum from the 1st to the 10th day of life, inclusive, with a total dose of 1 cc. of serum was followed by a precocious opening of the vagina and development of the uterus, but not by any significant increase in ovarian weight over that seen in littermate controls.

Daily vaginal smears were made from the first day of rupture of the vaginal membrane of littermate control rats and of the remaining rats which had been treated with antigonadotropic serum from the 1st to the 10th day of life, inclusive. Vaginal smears were taken from both groups until at least three vaginal estrous periods had been recorded for the control animals. The antihormone treated rats experienced vaginal estrous cycles beginning on the 22nd day to the 25th day of life and of these, thirteen rats had cycles comparable to those occurring in littermate control rats between the 45th and 50th day of age.

The serum used in the above experiments, as already noted, was obtained from rabbits repeatedly injected with sheep pituitary extracts. In order to eliminate the possibility that a non-specific substance in the serum might be the cause of the precocious sexual development, 0.5 cc. per day of normal rabbit serum was administered to 25 female rats from the 10th to the 19th day of life, inclusive. Eleven of these animals were killed on the 41st day of life to determine whether any gonadal stimulation followed cessation of treatment. The data in table 2 show that there is no significant difference between the average ovarian weight of the animals treated with normal rabbit serum and that of the untreated normal littermate females killed at the same age. The time of rupture of the vaginal membrane of the remaining 14 females that received normal rabbit serum was compared with that of untreated littermate control rats. The vaginas of the injected rats opened at an average of 48.2 days, while those of the untreated females opened at 47 days of age.

**DISCUSSION.** The data presented in this paper demonstrate that precocious gonadal activity, as evidenced by ovarian hypertrophy and premature establishment of the estrous cycles, follows treatment with antigonadotropic rabbit serum. The effect is not the result of the presence of gonadotropic substances in the antisera of the rabbits, for the ovaries of the animals injected with the antisera became atrophic while the serum was being administered. Likewise, the increase in size and function of the ovaries cannot be explained on the basis of the foreign protein of the serum since no acceleration or inhibition of gonadal development was obtained with normal rabbit serum. The explanation of the mechanism whereby gonadal stimulation follows antihormone administration is based upon the theory already suggested by us in a previous report (1). Briefly it is as follows: As a consequence of the antihormone injections the gonadotropic secretion of the animal is rendered ineffective, thereby causing an atrophy of the gonads. Continued administration of the antihormone is followed by further

atrophy of the gonads, which eventually results in a condition similar to that seen after hypophysectomy. As a result of the atrophied gonads there is an increase in the number of basophiles and the amount of gonadotropic secretion in the pituitary gland. The increased secretion of the basophilic pituitary, however, is prevented from reaching the gonads during the period of antihormone injection, but when the antihormone is withdrawn the hypersecretion of the gonadotropic hormone is no longer neutralized and the gonads are accordingly stimulated to a degree far beyond that of animals of the same age. Accompanying the increase in size and function of the ovary there is a gradual change in the castrate-like pituitary from a state of extreme basophilism to one of normal cell composition. Other experiments, however, showed that when the ovaries are removed at the end of antihormone treatment the basophilism found immediately after cessation of injections was maintained. In view of these results the gradual decrease in basophilism of the pituitary gland may be explained on the basis that the basophiles revert to chromophobes (7) and the ovarian hormones produced by the stimulated ovary prevent the differentiation of more than the normal number of chromophobes into basophiles.

#### SUMMARY

Hypersecretion of the gonadotropic hormone by the pituitary gland of immature female rats followed treatment with antigonadotropic serum. The hypersecretion resulted in a precocious development of the ovaries and premature occurrence of the cyclic variations in the vaginal smears of the treated rats after discontinuing the injections of the antigonadotropic serum. Extreme basophilism was found in the pituitary gland during treatment with the antigonadotropic sera but when the injections were discontinued the number of basophiles decreased to normal. This condition was accompanied by a marked increase in size and function of the ovaries. In control experiments the injection of rats with serum from rabbits not treated with gonadotropic extracts was without stimulatory or inhibitory effects upon the gonads.

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# PLASMA PROTEIN REPLACEMENT AFTER HEMORRHAGE IN DOGS WITH AND WITHOUT SHOCK

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Clinical and experimental studies of shock have emphasized the importance of the plasma proteins in maintaining an adequate plasma volume. In shock associated with trauma, burns and peritonitis, the plasma proteins are greatly diminished. Because the fluid in the traumatized, burned, or infected areas has been found to be rich in protein, the low plasma protein has been attributed primarily to the leakage of plasma protein from the blood stream into the tissues. It is obvious, however, that the quantity of plasma protein in the blood stream depends on the sum of two factors—i.e., the amount of protein added to the blood stream and the amount of protein lost from the blood stream. It seemed possible that failure of plasma protein production was an important factor in the marked decrease in the level of the plasma protein which is observed in shock. The purpose of this paper is to determine whether shock significantly affects protein regeneration.

Acute hemorrhage served as a means both for stimulating plasma protein production and for producing shock. The amount of protein that the dogs produced in the 12 hours immediately after venesection was determined *a*, when the hemorrhage was not severe enough to produce shock; and *b*, when the hemorrhage was severe enough to produce shock. In the latter experiments circulatory insufficiency was maintained by the removal of more blood whenever the circulation improved. The total plasma protein was measured before the bleeding and 12 hours after the first bleeding. The amount of protein produced was calculated by subtracting the total circulating plasma protein before hemorrhage from the sum of the plasma protein removed plus the circulating plasma protein 12 hours after the initial hemorrhage.

**METHOD.** The experiments were performed on normal unanesthetized adult mongrel dogs weighing 14 to 21 kgm. They ate a diet of cooked horse meat for at least 2 weeks before any observations were made, but were allowed no food for 36 hours before an experiment. Water was not restricted except for about one hour prior to and during the experiment.

The plasma volume was measured by the dye method (1, 2). Heparin was usually used as the anticoagulant, although a 1.6 per cent solution of potassium oxalate was used in 3 experiments. All samples of blood were taken without stasis. The total protein concentration in grams per cent was calculated from the protein nitrogen as determined by a standard micro-Kjeldahl technique (3). All nitrogen determinations were done in duplicate. The total circulating

plasma protein in grams was calculated by multiplying the protein concentration in grams per cent by the  $\frac{\text{plasma volume}}{100}$ .

The mean arterial pressure was measured in millimeters of mercury by inserting a needle into the femoral artery. In the majority of the experiments, the femoral artery was exposed under local anesthesia. In several experiments in which the circulation remained adequate, arterial punctures were performed without exposing the artery. The initial bleeding was made by puncture of the femoral artery on the unexposed side. Subsequent bleedings were made via the jugular or femoral veins. The blood was pooled in a flask containing a solution of 1.6 per cent potassium oxalate. Knowing the volume of fluid, the hematocrit reading and the protein concentration of the plasma-oxalate mixture, the amount of plasma protein removed was easily calculated. A correction was made for the plasma protein removed in sampling.

TABLE 1  
*Plasma protein replacement after a single large bleeding*

DOG	WEIGHT	INITIAL BLEEDING	BASAL				12 HOURS AFTER HEMORRHAGE				SUMMARY		
			Plasma volume	Plasma protein	Total circulating protein	Hematocrit reading	Plasma volume	Plasma protein	Total circulating protein	Hematocrit reading	Protein removed	Protein replaced	Percentage of total circulating protein replaced
	kgm.	cc.	cc.	grams per cent	grams		cc.	grams per cent	grams		grams	grams	
1	18.6	550	890	6.6	59	48	920	4.7	43	31	22	6	10
2	17	455	735	5.7	42	48	760	4.6	35	30	17	10	24
3	18.3	500	1120	5.3	59	39	1100	5.0	55	24	19	15	25
4	18	640	810	6.4	52	47	920	4.9	45	34	22	15	29
5	20.8	490	1200	5.7	68	41	1290	3.9	50	22	27	9	13

Shock was produced by the rapid removal of blood from the femoral artery. It was maintained by further bleeding whenever the animal showed signs of improvement. The details of the method and the criteria used for determining the presence of shock are given below.

**RESULTS.** *Plasma protein production after hemorrhage without shock.* Plasma protein production was studied in 11 dogs in which the bleeding was not sufficient to produce any marked change in the dog's appearance or behavior. After each bleeding (with the exception of dog 6), an equal amount of normal saline was given intravenously. Some of the dogs had little or no fall in mean arterial pressure. In others there was a decrease of 30 to 40 mm. Hg, but the animals remained active, showed no disturbance of gait, and maintained good muscle tone. In 5 dogs a single large bleeding was done at the beginning of the experiment. A 30 cc. sample of blood for plasma volume determination was removed one hour later. The data are summarized in table 1. These dogs produced from 6 to 15 grams of plasma protein in 12 hours. This amounted to 10 to 29 per cent of their

total circulating plasma protein. In 6 dogs the large initial hemorrhage was followed up by repeated small bleedings throughout the day. In this way much larger amounts of protein were removed without noticeably impairing the circulation. The data are given in table 2. In the 12-hour period these dogs produced from 5 to 15 grams of plasma protein, or from 10 to 30 per cent of their total circulating plasma protein. The amount of protein produced in the 12-hour period could not be correlated with the amount of plasma protein removed.

In 4 of the dogs who had a single large bleeding (dogs 1, 2, 3, 4), the amount of protein produced in the second 12 hours after hemorrhage was also measured.

TABLE 2

*Plasma protein replacement after hemorrhage with an adequate circulation and with shock*

DOG	EXPERIMENT	DATE	BASAL				12 HOURS AFTER HEMORRHAGE				SUMMARY			PHYSIOLOGICAL SALINE	AVERAGE MEAN BLOOD PRESSURE
			Plasma volume	Plasma protein	Total circulating protein	Hematocrit reading	Plasma volume	Plasma protein	Total circulating protein	Hematocrit reading	Protein removed	Protein replaced	Percentage of total circulating protein replaced		
			cc.	gms. per cent	gms.		cc.	gms. per cent	gms.		gms.	gms.		cc.	mm. Hg
6	I*	6-12-41	960	6.3	60	50	835	4.8	40	39	35	15	25	120	70
	II*	7- 3-41	1025	6.5	67	49	780	4.7	37	35	31	1	1	0	45
7	I	5-20-41	690	6.3	44	55	710	4.9	35	36	22	13	30	700	105
	II	6-26-41	730	6.6	48	53	665	3.5	23	28	26	1	2	700	45
8	I	7- 1-41	925	6.1	56	47	1090	4.2	46	25	25	15	27	1100	105
	II	5-28-41	870	6.6	57	53	670	4.6	31	31	29	3	5	0	65
9	I	7-18-41	950	5.6	53	43	930	4.3	40	22	24	11	21	1140	95
	II	6-19-41	970	5.9	57	48	730	4.1	30	22	32	5	9	250	55
10	I	9- 4-41	745	6.5	48	50	845	4.3	36	32	17	5	10	550	80
	II	7-15-41	690	5.8	40	54	560	3.3	18	28	23	1	3	1160	45
11	I	6-12-41	870	5.9	51	45	980	4.0	39	23	23	11	22	1000	85
	II	7-22-41	810	5.9	48	54	640	4.2	27	29	31	10	21	400	45

\* I = hemorrhage without shock.

\* II = hemorrhage with shock.

In the first 12 hours these dogs made 6, 10, 15 and 15 grams. In the second 12 hours they made 9, 8, 10 and 11 grams respectively. In the same 4 dogs measurements were also made at approximately one hour after the initial hemorrhage. They had produced 0, 3, 2 and 1 gram respectively. These figures are too small to be significant in view of the errors in the method. They do show, however, that no large amount of protein entered the blood stream immediately after hemorrhage.

*Protein regeneration after hemorrhage with shock.* Shock from hemorrhage was produced in 6 unanesthetized dogs. The ability of 3 of these dogs to regenerate protein after hemorrhage without shock had been tested from 3 to 5 weeks pre-



viously. In 3 dogs the ability to produce protein after hemorrhage without shock was tested from 3 to 7 weeks after the shock experiment.

Following the initial hemorrhage, the arterial pressure usually dropped to between 20 and 30 mm. Hg. The animals were restless; they voided and defecated involuntarily. In a short time the arterial pressure began to rise and more blood had to be removed. After several hours the arterial pressures became stabilized at a low level which varied from dog to dog. The average arterial pressure was obtained by charting the mean arterial pressure for the 12-hour period. It ranged from 45 to 65 mm. Hg. The dogs were very weak, apathetic, and at times stuporous. During much of the time they were too weak to raise their heads. Chemically the circulatory insufficiency was demonstrated by a rising non-protein nitrogen and a decrease in the  $\text{CO}_2$  combining power. In the control experiments, the non-protein nitrogen remained normal and the  $\text{CO}_2$  combining power showed little change.

As other investigators have noted, there was no absolute correlation between the mean arterial pressure and the general condition of the dog. Some dogs with a mean pressure of 45 appeared stronger and more alert than others did at a mean pressure of 65. In general, an attempt was made to keep the mean arterial pressure below 50. The best criterion of the condition of the circulation seemed to be the response to the removal of a small amount of blood. If the removal of 20 cc. of blood produced a precipitous fall in arterial pressure, the animal was in a critical state. If it produced no change in arterial pressure, the animal could usually stand further bleeding.

After the first 3 hours little or no blood had to be removed. The animals had to be watched closely because a sudden marked fall in arterial pressure, not produced by further bleeding, frequently occurred, and unless saline was given, they died from cerebral anoxemia. Some of the dogs received as much saline in the course of 12 hours as they did in the hemorrhage experiments without shock. The saline would not restore the circulation to normal after it had been inadequate for several hours, but it would produce sufficient improvement to prevent the death of the animal. All the dogs recovered.

In 5 of the 6 experiments, the dogs in shock produced very little protein in the 12-hour period. The sixth dog produced as much protein as he had after hemorrhage without shock. The average mean arterial pressure in this dog was 45 mm. Hg. It was noted in the protocol, however, that in spite of the low arterial pressure, this dog had been more alert and active than the other dogs. The data are summarized in table 2.

COMMENT. The determination of the plasma volume seemed as satisfactory in the dogs with an inadequate circulation as in normal dogs. Fifteen minutes were always allowed for complete mixing of the dye. In general, the slope of dye disappearance curve was approximately the same before and after the production of shock. The values obtained for the plasma volume and for the total circulating protein seemed reasonable. One hour after hemorrhage the plasma volume was decreased and the total circulating protein was approximately equal to the prehemorrhage total circulating protein minus the amount of protein removed.

Twelve hours after hemorrhage, protein replacement was well under way and, with one exception, the plasma volume had increased to or beyond its prehemorrhage level. Twenty-four hours after hemorrhage a further increase in plasma volume and in total circulating protein had occurred. In the dogs maintained in shock, the plasma volume at the end of 12 hours was always smaller than the original plasma volume. It is possible that the plasma volume of the dogs in shock was smaller during the earlier part of the 12-hour period. During the first 3 to 6 hours, a slight increase in plasma volume produced a marked rise in arterial pressure. After the circulatory insufficiency had been present approximately 6 hours, increasing the plasma volume by saline frequently produced little change in arterial pressure. Freeman (4) has reported similar observations.

Fasting dogs with an adequate circulation vary considerably in their ability to replace plasma protein lost by hemorrhage. There was no correlation between the amount of protein produced and the level of the plasma protein before hemorrhage, or between the amount of protein produced and the amount of protein removed. The diets previous to the control period and the age of the dogs were not known. In the 24-hour period after hemorrhage, protein was apparently added to the blood stream at a fairly constant rate. Three of the 4 dogs tested replaced slightly greater amounts of protein in the first 12 hours than in the second 12 hours after hemorrhage. Too few experiments were done to determine whether this difference was significant.

The rate of mobilization of the protein stores recorded here is in accord with the concept of Madden and Whipple (5), that the greater portion of the protein reserve is not stored as preformed plasma protein. In fact, these experiments demonstrated no measurable reserve of preformed plasma protein which could be added to the blood stream in a few minutes. There was no evidence of a rapid out-pouring of protein in the first hour after hemorrhage. Similar observations have been made after hemorrhage in normal human subjects (6). This is further supported by the observation that 3 of the dogs in shock added only 1 gram of protein to the plasma during a 12-hour period. This question of a small reserve of preformed protein is of considerable importance, because even in the absence of increased capillary permeability, changes in plasma protein concentration would not necessarily reflect changes in the plasma volume if there were a reserve of preformed plasma protein which could be added to the blood stream in a few minutes. Smith, Belt and Whipple (7) have reported a rapid replacement of serum proteins in the first 15 minutes after the replacement of a large portion of the blood by an equal quantity of a red cell Locke's solution mixture. This resulted in an increase in serum protein concentration of about 0.5 gram per cent. As the hematocrit reading showed little rise during this period, and as the rise in fibrin concentration was not proportionally as great as the increase in the serum protein concentration, they concluded that the rapid replacement of serum protein during the first 15 minutes following the exchange indicated some reserve supply of serum proteins probably held in the body cells. In our experiments with dogs and with normal human subjects after bleeding, physiological saline has left the blood stream very rapidly, and the replacement of a quantity of

saline equal to the volume of blood removed has failed to restore the plasma volume to the prehemorrhage level. In the experiments of Smith, Belt and Whipple, the hematocrit readings increased from an average of 53 to an average of 56 in the first 15 minutes. If this increase in hematocrit reading resulted from a decrease in plasma volume, it would account for about one-half of the observed rise in serum protein concentration. In these experiments, one would not have expected the hematocrit reading to parallel the decrease in plasma volume, because a considerable number of traumatized red cells were being destroyed, as shown by the presence of hemolysis in many of the samples and by the subsequent marked fall in the hematocrit reading. In the first 15 minutes, the average increase in serum protein concentration was 23 per cent. At the same time, the fibrin increased an average of 14 per cent. These do not seem significant differences, because an average increase of only 0.01 gram per cent of fibrin would have resulted in a parallel increase in serum protein concentration during the first 15 minutes after the replacement.

The protein replacement in the dogs with shock was significantly less than in the dogs in which the hemorrhage was not accompanied by shock. This is based on the assumption that the protein added to the plasma remained in the blood stream and did not pass into the tissues because of altered capillary permeability. It has been recently demonstrated that the capillaries in shock due to hemorrhage are not abnormally permeable. Price et al. (8) injected a large amount of blue dye, T-1824, into the veins of dogs. This enabled them to observe directly abnormal leakage of dye, and presumably protein, into the tissues. They stated: "Although local staining of tissues was pronounced in areas subjected to trauma of different sorts, no abnormal staining of tissues in general was observed in animals dying of post-hemorrhagic shock."

The data indicate that failure of the circulation was the primary factor which prevented normal plasma protein replacement in the dogs in shock. The low protein replacement in the dogs in shock was not caused by lack of adequate fluid intake, because in 2 dogs as much or more saline was administered during the shock experiment as during the simple hemorrhage experiments. The stimulus for protein replacement should have been as great in the dogs in shock as in the dogs not in shock, because the protein concentration was greatly reduced in both groups of experiments. The loss of red cells was not the determining factor, because the hematocrit levels at the end of the 12-hour period were not very different in the two types of experiments. The data in table 2 show that in dogs 6, 7 and 8 there was no great difference in the amount of protein removed in the simple hemorrhage and in the shock experiments. The speed with which the blood was removed, rather than the total amount removed, determined whether shock developed.

After simple hemorrhage the dog first increases his plasma volume by dilution with protein-poor fluids. This restores the circulation to an adequate level and protein replacement proceeds fairly rapidly. Studies in man have shown a similar chain of events (6). In the dog which is kept in shock by repeated small hemorrhages, the dilution with protein-poor fluid occurs, but the further loss of

plasma by bleeding prevents the circulation from becoming adequate for protein regeneration. After the original hemo-dilution has occurred, it is difficult to maintain the plasma volume at a normal level by the administration of normal saline because of the low colloid osmotic pressure of the blood. Therefore, recovery is very slow unless plasma or some other fluid with similar osmotic properties is administered.

It is believed that these experiments which demonstrate the difficulty in protein replacement with a poor circulation offer an adequate explanation for the clinically observed fact that patients with hemorrhage who maintain a good circulation can lose a tremendous quantity of plasma protein and maintain an adequate plasma volume, while patients in certain types of shock have great difficulty in restoring the volume to normal.

#### SUMMARY

1. Plasma protein replacement after acute hemorrhage, which was not severe enough to produce shock, has been studied in unanesthetized, fasting dogs. One hour after hemorrhage little protein had been added to the plasma. Twelve hours after hemorrhage from 6 to 15 grams of plasma protein had entered the blood stream. This amounted to 10 to 29 per cent of the total prehemorrhage circulating plasma protein. Plasma protein replacement proceeded at the same or at a slightly lower level in the second 12-hour period after hemorrhage.

2. These experiments showed no evidence of a reserve of preformed plasma protein which can enter the blood stream in the first few minutes after hemorrhage.

3. In 6 unanesthetized, fasting dogs, shock was produced and maintained by hemorrhage. Plasma protein replacement was measured over a 12-hour period. Shock usually greatly retarded plasma protein replacement. Dogs which had replaced 15, 13, 15, 11, 5 and 11 grams of plasma protein in a 12-hour period after hemorrhage without shock replaced only 1, 1, 3, 5, 1 and 10 grams respectively in a 12-hour period when they were in shock.

4. The slow rate of plasma protein production with an inadequate circulation explains why the body has great difficulty in restoring the plasma volume to normal in many cases of shock.

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# THE RELATION OF THE PITUITARY, THYROID AND ADRENAL GLANDS TO THE MAINTENANCE OF NORMAL SERUM ALBUMIN AND GLOBULIN LEVELS<sup>1, 2</sup>

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Goldberg (1, 2) has reported that hypophysectomy in dogs produces a fall in serum albumin and a compensating increase in serum globulin concentration. Administration of desiccated thyroid to such dogs caused a return of the globulin toward the normal level but had very little effect on the serum albumin concentration. These findings led Goldberg to suggest that the decreased thyroid activity following hypophysectomy is responsible for the changes in serum protein levels.

Before this work came to our attention we (3) had found similar changes following hypophysectomy in the monkey. Transferring our investigations to the rat, we found that in this species hypophysectomy produces serum protein changes similar to those in the dog and the monkey. In the rat, thyroid medication prevents the increase in globulin level but has little if any effect on the albumin level, confirming Goldberg's findings in the dog. These results indicate that some factor, other than the thyroid gland, is responsible for the post-hypophysectomy albumin changes. For reasons to be discussed below, it appeared probable that the adrenal cortical atrophy which follows pituitary ablation might be related to the marked decrease in serum albumin concentration. Attempts were therefore made to study the rôle of the adrenal cortex and to define more clearly the action of the thyroid with respect to maintenance of the serum protein levels. Since a partial inanition occurs after hypophysectomy (4) and since it is known (5) that in certain species alterations of serum protein levels are produced by severe protein inanition, a study of the effect of reduced food intake on the serum protein levels of the rat was also made.

The results of these experiments, forming the basis of this report, indicate that in the rat the maintenance of the serum globulin level is associated with the degree of thyroid activity while the maintenance of the albumin level appears to depend on adrenocortical function. The inanition which follows hypophysectomy, also a factor in the alteration of the serum protein levels, appears to be of lesser importance than does the decreased activity of the adrenal cortex and of the thyroid gland.

EXPERIMENTAL. Adult rats, in most experiments males, of the Long-Evans

<sup>1</sup> Aided by a grant from the Rockefeller Foundation, administered by Dr. P. E. Smith.

<sup>2</sup> A preliminary report of this work was made before the thirty-fifth annual meeting of the American Society of Biological Chemists at Chicago, April 19, 1941 (*J. Biol. Chem.* 140: p. lxxvi, 1941).

strain, were used. The animals were maintained under the usual conditions prevailing in our stock colony. The regular adequate stock diet used for our colony, as well as water, was available at all times except as specified below.

The completeness of pituitary removal was verified by microscopic examination of serial sections of the sella. Thyroidectomized animals were examined under the dissecting microscope for thyroid remnants and if any suspicious tissue was seen serial sections of the thyroid region were made and studied. The one case of incomplete thyroidectomy was discarded from the series. In the adrenalectomized animals a gross search at autopsy revealed no cortical tissue but it is realized that accessory cortical tissue might have been present and not seen.

Whenever treatment was given it was begun immediately after operation and was administered by subcutaneous injection daily until the time of removal of blood, in most cases 18 to 21 days post-operatively. Blood was taken directly from the heart which was exposed under amytal anesthesia. A small portion of each blood sample was heparinized and used for hematocrit determination (6). The remainder of the blood was allowed to clot, the serum separated and used for determination of total protein, albumin and non-protein nitrogen. Semi-micro Kjeldahl technic was used for all nitrogen determinations. Albumin filtrates were prepared by the method of Campbell and Hanna (7). The nitrogen values, after correction for non-protein nitrogen, were converted to protein by use of the usual factor 6.25 and globulin was calculated as the difference between total protein and albumin.

**RESULTS.** The data obtained from untreated normal and hypophysectomized rats are given in table 1, A, B, D, E. The most striking changes resulting from the pituitary ablation are the decrease in serum albumin and the increase in serum globulin. The net result is a decrease in total protein which is relatively slight but statistically significant and a fall in the ratio of albumin to globulin to almost half the normal value.

Following hypophysectomy the animals lost a considerable proportion (males 24.7 per cent; females 14.4 per cent) of the pre-operative body weight. To simulate this weight loss as closely as possible a group of female rats of similar age and weight was subjected to a reduced food intake so that the weight loss (23.6 per cent of the original) approximated in duration and intensity that suffered by the hypophysectomized animals. The changes in serum albumin, globulin, NPN and A/G ratio (table 1, C) are in the same direction but of much lesser magnitude than after hypophysectomy.

The results obtained from thyroidectomized rats are given in table 1, F. The slight decrease in serum albumin is of doubtful significance ( $P = 0.05$ ).<sup>3</sup> The globulin fraction, however, increased to an even greater extent (38.6 per cent over the normal level) than after hypophysectomy (29.1 per cent over the normal

<sup>3</sup> The value designated as "P" represents the probability that the difference between two means is due to random sampling (21). A probability of 0.05 (five chances in 100 that random sampling is responsible for the difference) is frequently selected as a criterion of significance.

TABLE 1  
Effect of various treatments on serum protein levels in the rat

TREATMENT	NO.	AGE AT BLEEDING	DURING EXP'T.		CELL VOLUME	NON-PROTEIN NITROGEN	TOTAL PROTEIN	ALBUMIN	GLOBULIN	ALBUMIN GLOBULIN
			Time	Wt. chg.						

Females										
A. Normal.....	11	88-137	days	per cent	per cent	mgm. per cent	per cent	per cent	per cent	ratio†
B. Hypsect., untreated.....	13	94-136	13-43	-14.4 ± 2.0	38.7 ± 0.6	34.1 ± 1.1	5.71 ± 0.08	3.70 ± 0.04	2.01 ± 0.09	1.88 ± 0.09
Per cent change from normal.....					32.0 ± 1.4	44.3 ± 2.0	5.30 ± 0.12	2.63 ± 0.06	2.67 ± 0.14	1.02 ± 0.05
P* (vs. normal).....					-17.3	+29.9	-7.2	-28.9	+32.8	-45.8
C. Intact, partially starved.....	11	109-225	13-41	-23.6 ± 1.0	<0.01	<0.01	0.02	<0.01	<0.01	<0.01
Per cent change from normal.....					37.3 ± 1.0	35.5 ± 1.6	5.70 ± 0.15	3.35 ± 0.12	2.35 ± 0.15	1.49 ± 0.12
P* (vs. normal).....					-3.6	+4.1	-0.2	-9.5	+16.9	-20.7
					0.30	0.40	0.90	0.02	0.05	0.02

Males										
D. Normal.....	31	100-164	19-21	+5.9 ± 2.0	43.2 ± 0.6	36.2 ± 1.2	5.88 ± 0.08	3.69 ± 0.06	2.20 ± 0.07	1.73 ± 0.06
E. Hypsect., untreated.....	30	112-162	19-21	-24.7 ± 0.9	41.3 ± 0.9	44.9 ± 0.9	5.60 ± 0.08	2.76 ± 0.06	2.84 ± 0.08	1.00 ± 0.04
Per cent change from normal.....					-4.4	+24.1	-4.8	-25.2	+29.1	-42.2
P* (vs. normal).....					0.09	<0.01	0.02	<0.01	<0.01	<0.01
F. Thyrect., untreated.....	11	103-164	20-44	+10.6 ± 5.7	40.9 ± 1.4	38.2 ± 1.0	6.52 ± 0.11	3.47 ± 0.09	3.05 ± 0.15	1.17 ± 0.08
Per cent change from normal.....					-5.3	+5.5	+10.9	-6.0	+38.6	-32.4
P* (vs. normal).....					0.10	0.40	<0.01	0.05	<0.01	<0.01
G. Hypsect., thyroxin treated 10-20 µg. daily.....	16	124-140	20-21	-29.9 ± 1.3	46.1 ± 0.9	42.5 ± 0.7	5.14 ± 0.08	2.93 ± 0.06	2.21 ± 0.07	1.35 ± 0.06
Per cent change from normal.....					+6.7	+16.3	-12.6	-20.6	0.00	-22.0
P* (vs. untreat. hypsect.).....					<0.01	0.04	<0.01	0.06	<0.01	<0.01
H. Adren., untreated.....	6	132-161	19-21	-18.1 ± 0.7	41.0 ± 1.5	71.3 ± 4.6	5.70 ± 0.14	3.09 ± 0.15	2.61 ± 0.06	1.19 ± 0.07
Per cent change from normal.....					-5.1	+97.0	-3.1	-16.3	+18.6	-31.2
P* (vs. normal).....					0.15	<0.01	0.40	<0.01	0.02	<0.01
I. Hypsect., DCA† treated 3-5 mgm. daily.....	12	122-143	18-21	-19.8 ± 1.3	40.6 ± 0.5	37.7 ± 1.0	5.58 ± 0.13	3.02 ± 0.07	2.56 ± 0.13	1.22 ± 0.08
Per cent change from normal.....					-6.0	+4.2	-5.1	-18.2	+16.4	-29.5
P* (vs. untreated hypsect.).....					0.60	<0.01	0.90	0.02	0.03	0.01
P* (vs. normal).....					0.01	0.40	0.04	<0.01	0.01	<0.01
Per cent change from normal (corr.)§.....					0.00	+16.0	+5.5	-9.5	+29.1	-29.5
J. Hypsect., C.E.‡ treated 1-4 cc. daily.....	17	125-157	18-21	-26.0 ± 0.9	42.4 ± 0.9	45.6 ± 0.9	6.11 ± 0.13	3.33 ± 0.07	2.78 ± 0.13	1.26 ± 0.08
Per cent change from normal.....					-1.9	+26.0	+3.9	-9.8	+26.4	-27.2
P* (vs. untreated hypsect.).....					0.45	0.60	<0.01	<0.01	0.70	<0.01
P* (vs. normal).....					0.40	<0.01	0.10	<0.01	<0.01	<0.01
K. Intact Silbestrol 1 mgm. daily.....	12	121-157	21	-25.9 ± 1.9	33.6 ± 1.0	39.5 ± 1.1	6.22 ± 0.10	4.09 ± 0.09	2.13 ± 0.06	1.94 ± 0.07
Per cent change from normal.....					-22.2	+9.1	+5.8	+10.8	-3.2	+12.1
P* (vs. normal).....					<0.01	0.10	0.02	<0.01	0.50	0.05

The  $\pm$  values are for the mean deviation of the mean, calculated as  $\bar{E}_m = \sqrt{\frac{\sum d^2}{n(n-1)}}$ .

\* P expresses the probability that the difference between two means is due to random sampling (see footnote 3).

† The values given for A/G ratio are the means of the individual A/G ratios rather than the ratio of the mean albumin to mean globulin.

‡ DCA = desoxycorticosterone acetate. C.E. = adrenal cortical extract.

§ These values obtained by correcting for the hemodilution indicated by the decreased hematocrit.

level). The implications of these results are reinforced by the results obtained from animals treated with 10 to 20  $\mu$ g. of thyroxin daily from the time of hypophysectomy until bleeding (table 1, G). Although the thyroxin treatment did not prevent the decrease in serum albumin which followed hypophysectomy, the increase in the globulin fraction was entirely prevented.

Because changes in thyroid activity appear to have very little effect on the serum albumin level, a series of experiments was made to investigate the relation of adrenal cortical action to the serum proteins. Although complete adrenalectomy is almost impossible in the adult rat, a small group of animals were adrenalectomized with the hope that the chronic partial adrenal insufficiency might show some effect. It should be emphasized that a considerable proportion of such animals died before the routine three weeks postoperative period had elapsed. It seems justifiable to assume that these suffered the highest degree of cortical insufficiency. Therefore, any serum protein changes found in the surviving rats are probably representative of only the milder forms of cortical insufficiency. It may be noted (table 1, H) that the animals lost considerable weight and yielded low serum sodium (average 298.4 mgm. per cent) and high serum NPN values. Surprisingly, the hematocrit readings showed no significant change from normal. The changes in serum protein levels are similar in direction to those following hypophysectomy but are of considerably lesser magnitude. As compared to values obtained from intact rats, the albumin is significantly decreased ( $P = < 0.01$ ) and the globulin increased ( $P = 0.02$ ).

In order to evaluate more completely the effect of the cortical insufficiency which follows hypophysectomy, two series of hypophysectomized rats were given cortical replacement therapy daily throughout the post-operative period. One group (table 1, I) was given desoxycorticosterone acetate (3.0-5.0 mgm. daily) while the other group (table 1, J) was treated with adrenal cortical extract (1.0 to 4.0 cc. daily).<sup>4</sup> The DCA treatment did not prevent the weight loss which follows hypophysectomy. However, it did partially but significantly ( $P = 0.02$ ) prevent the decrease in serum albumin level which occurs in untreated hypophysectomized animals. The protection against serum globulin increase was not significant ( $P = 0.08$ ). It should be noted that the hematocrit reading of the DCA treated animals is significantly below that of the normal rats ( $P = 0.01$ ). If the assumption is made that this decrease in hematocrit is due to hemodilution and an appropriate correction is made, it may be seen (table 1, I) that the post-hypophysectomy change of albumin concentration is largely prevented (9.5 per cent below normal) by the DCA while the globulin change (to 29.1 per cent above normal) is almost identical with that of untreated hypophysectomized rats.

Treatment with cortical extract did not prevent the post-hypophysectomy

<sup>4</sup> The authors wish to express their thanks for generous supplies as follows: For adrenal cortical extract to Wilson Laboratories, through Dr. David Kline, and to the Upjohn Company, through Dr. G. F. Cartland. For desoxycorticosterone acetate to Ciba Pharmaceutical Products, through Dr. R. C. Mautner, and to the Schering Corporation, through Dr. Erwin Schwenk. For stilbestrol to E. R. Squibb and Sons, through Dr. J. A. Morrell.



weight loss nor the increase in serum NPN level. However, the decrease in serum albumin was largely prevented ( $P = < 0.01$ ) while the globulin increase was not significantly different ( $P = 0.70$ ) from that of untreated animals, the net result being a total protein value higher than that of either normal or hypophysectomized rats.

Stilbestrol, administered to normal rats as a corticotropic agent (1.0 mgm. daily) produced adrenal enlargement and hyperemia as previously reported by Selye (8) and many others. Despite the rather severe inanition suffered by these animals (table 1, K) the serum albumin level rose considerably above the normal value while the globulin level remained almost constant. The hematocrit value fell considerably.

**DISCUSSION.** Following hypophysectomy the adult rat suffers a pronounced loss of body weight which is associated with a decreased food intake (4) and an impaired absorption from the gastro-intestinal tract (9, 10). That the resultant inanition is at most only partially responsible for the serum protein changes is shown by the finding that in intact rats inanition of somewhat greater severity than that following hypophysectomy produced less than half as much change in serum protein levels. This is confirmed by the data obtained from normal rats treated with stilbestrol. Such treatment causes a marked loss of body weight which is almost certainly a result of the decreased food consumption for Ingle (11) has shown that forcibly feeding such animals produces a weight gain instead of a loss. Despite the severe inanition suffered by our stilbestrol treated rats, the serum albumin concentration actually increased while that of the globulin remained constant. It may also be noted that hypophysectomized rats treated with adrenal cortical extract suffered as severe a weight loss as did the untreated operated animals. The serum albumin concentration, however, decreased only slightly thus confirming the fact that during short experimental periods the changes in serum protein levels are not necessarily proportional to the degree of inanition.

Our results support Goldberg's suggestion (1, 2) that the thyroid inactivity which follows hypophysectomy is responsible for the increase in the serum globulin levels. However, neither his data nor ours justify the belief that the lack of thyroid activity is responsible for the change in albumin concentration. Administration of thyroxin to hypophysectomized rats has little if any effect on the decrease in albumin level. Nor does removal of the thyroid produce a pronounced albumin decrease as occurs after hypophysectomy. It seems quite definite, therefore, that the thyroid is in some way involved in the maintenance of the serum globulin level but exerts little, if any, control over the serum albumin metabolism.

The adrenal cortex is known to have an important influence on salt and water metabolism and to exercise control over the distribution of body fluids and the maintenance of blood volume (12, 13). It is also known to be intimately concerned with the intermediary metabolism of proteins (14). This knowledge, taken together with the fact that the serum albumin is one of the most important factors in the maintenance of blood osmotic pressure (5, 15) and, therefore, of

blood volume, indicates the possibility of a direct relationship between adrenocortical activity and serum albumin metabolism.

Treatment of hypophysectomized rats with cortical substances yields evidence supporting this hypothesis. Thus, administration of adrenocortical extract largely prevents the fall in serum albumin which occurs in untreated animals. The increase in serum globulin, however, is not prevented. When desoxycorticosterone is used for replacement therapy almost identical results are obtained if the lowered hematocrit is taken into account. Such a correction of values for hemodilution is not illogical in the light of the well known blood diluting effect of desoxycorticosterone.

The treatment of hypophysectomized rats with the cortical substances did not completely maintain the serum albumin concentration at its normal level. It must be remembered that the dosages used, as well as the mode and spacing of the injections, may have been far from optimal. Furthermore, some other of the substances now known to be elaborated by the adrenal cortex may be more effective than the substances used in the present experiments. In this connection it is well to recall the different potencies of these substances with respect to carbohydrate metabolism, salt and water metabolism, etc. (14, 16, 17, and others).

The treatment of normal rats with stilbestrol provides further evidence indicating control of serum albumin metabolism by the adrenal cortex. Stilbestrol appears to be a potent stimulant to the adrenal cortex (8, 11, and many others) and might be expected, therefore, to produce an increase in the serum albumin concentration. Such an increase would be of limited magnitude because it, *per se*, would tend to cause a proportional hemodilution. The results obtained were as anticipated, the albumin level of the treated animals being slightly (10.8 per cent) but significantly ( $P = 0.01$ ) above the normal level. The globulin level did not change appreciably as would be expected if the adrenal cortex controls albumin but not globulin metabolism. The hematocrit value was markedly lower than normal, possibly a consequence of decreased hemopoiesis (18, 19) or of hemodilution induced by the increased concentration of the serum albumin.

The results obtained from the small group of adrenalectomized rats do not entirely fit into the general picture. In this case decreased albumin and increased globulin levels were found, indicating no preferential control. However, as noted in a previous section of this paper, the cortical insufficiency exhibited by the animals which survived the entire experimental period is probably very mild. Furthermore, the animals apparently suffered a considerable loss of appetite as reflected by the loss of body weight. It is not improbable, therefore, that the observed changes are the combined effect of inanition and mild cortical insufficiency. The globulin change is very nearly the same as that suffered by animals subjected only to inanition. The albumin decrease is somewhat greater than that produced by inanition alone and may be interpreted as the combined effect of the inanition plus the mild cortical insufficiency. In this connection may be mentioned the results obtained from two small groups of adrenalect-

tomized rats, one treated with desoxycorticosterone acetate (2.5 mgm. daily) and the other drinking 1 per cent NaCl solution instead of tap water. In each case the animals showed a small weight gain as contrasted to the weight loss suffered by untreated adrenalectomized rats. Other changes shown by the untreated animals were completely prevented with the exception of the decrease in serum albumin level in the case of the group drinking 1 per cent NaCl. The albumin level of these rats was 8.1 per cent below normal ( $P = 0.03$ ). This may be interpreted to mean that the DCA therapy completely replaced the lost adrenocortical function but that increasing the NaCl intake, while preventing the loss of appetite, did not replace the adrenal cortex insofar as maintenance of serum albumin level is concerned.

The concept of maintenance of serum albumin by adrenocortical influence is not inconsistent with the recent findings of Long (14, 20) which indicate that the adrenal cortex controls general protein catabolism. According to Long, adrenocortical activity favors breakdown of body protein to amino acids of which a portion are converted to carbohydrate. It is not unlikely that another portion of these newly formed amino acids may be used to replenish or replace other vital substances of which serum albumin is representative.

The authors wish to express their thanks to Dr. G. K. Smelser for performing the thyroidectomies reported in this paper and to Mrs. Elizabeth Wolfe for valuable technical assistance.

#### SUMMARY AND CONCLUSIONS

1. Hypophysectomy in rats produces a fall in serum albumin and an increase in serum globulin with a slight resultant decrease in total serum protein. These changes are reproduced to only a slight extent by inanition similar in duration and intensity to that suffered by the hypophysectomized rats.

2. Thyroidectomy simulates hypophysectomy in so far as increase of serum globulin is concerned. There is very little, if any, effect on the serum albumin level. Conversely, treatment of hypophysectomized rats with thyroxin prevents the increase in serum globulin but does not inhibit the decrease of the serum albumin level.

3. Treatment of hypophysectomized rats with adrenal cortical extract or with desoxycorticosterone, in the doses used, to a large extent prevents the decrease in albumin level with little or no effect on globulin level.

4. Stilbestrol, administered to intact rats, causes an increase in serum albumin level, presumably via the adrenal cortex which is markedly stimulated. There is no effect on the globulin level.

5. The above findings lead to the tentative conclusion that in the rat the serum albumin level is maintained under adrenocortical influence while the serum globulin level is associated with activity of the thyroid gland. Following hypophysectomy, a change in both fractions occurs because of the decrease in both adrenocortical and thyroid activity.

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# LINEAR RELATIONSHIP BETWEEN THE CIRCULATING RED CELL MASS AND THE VENOUS HEMATOCRIT AS DETERMINED WITH RADIOACTIVE IRON

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When iron tagged with radioactive isotope<sup>1</sup> is administered under such conditions that the absorbed iron is utilized immediately for red cell formation, the concentration of tagged iron in the blood stream may remain quite constant for many months (3). Exchange of the radio iron of the blood stream does not occur with other iron of the body (5) and it seems that the isotope once incorporated into the red blood cell remains there until the cell undergoes disintegration (1, 3). Thus, under conditions under which no destruction of the labeled cells is occurring, it would seem that the total labeled iron in the circulation, once established, remains constant.

It is therefore possible to study the relationship between circulating red cell mass and jugular hematocrit at various hematocrit levels by using the radio iron-labeled-donor cell method described in detail elsewhere (6, 7). This method consists essentially of a transfusion from a donor animal of a few milliliters of blood containing an accurately known amount of iron built into red cells, and then by measuring the radioactivity of subsequent blood samples to determine the dilution that has occurred.

It is to be expected that increased jugular or venous hematocrit indicates an increased mass of red blood cells and hemoglobin in the experimental animal or patient. There are data on one dog (3) to indicate that the circulating red cell mass is quite accurately proportional to the jugular hematocrit. However, this donor cell technique affords a method for determining on an individual animal the red cell mass at a large number of widely different hematocrit levels. Data are therefore here presented on results of 24 pairs of determinations of hematocrit and cell mass on three dogs with hematocrit readings varying from 11 to 57 per cent.

**METHODS.** The animals used were all adult mongrel dogs which had been vaccinated against distemper. Their care has been described elsewhere (8). Diet consisted of hospital table scraps.

Plasma volume was done by a modification of the Brilliant Vital Red dye procedure (9). Samples were taken from the jugular veins on the same side of the neck as was used for the dye injection, the needle being flushed with blood occasionally. Stasis was avoided. Amounts of dye were injected such that the final dilutions resulted in readings of both standard and unknown in the colori-

<sup>1</sup> We are indebted to the members of the Radiation Laboratory at Berkeley, California, and in particular to Dr. E. O. Lawrence and Dr. M. D. Kamen for the radioactive iron used.

meter within 10 per cent of one another, these amounts being determined previously for each dog. A single dyed sample was taken at the end of four minutes after injection of the dye.

Hematocrits were determined on the samples taken above, the blood being taken in 2 ml. of isotonic (1.4 per cent) sodium oxalate in 15 ml. centrifuge tubes and spun at about 2700 rpm. for 35 minutes.

Red cell circulating volumes were determined as follows: An initial sample of 30 ml. of blood was drawn into 5 ml. of isotonic oxalate. Twenty-five milliliters of blood containing citrated radioactive red cells from a donor were then given by vein through the same needle within thirty seconds' time. After 15 minutes during which the animal was permitted to stand in a cage, another 30 ml. of blood were drawn as before. The blood was divided in each case so there were triplicate samples for determination of isotope activity. The aliquots were centrifuged for 35 minutes and the hematocrits of each read and averaged. The red cells were then ashed and the iron separated for electroplating (3) and activity determinations (3, 4). An aliquot representative of the injected blood was treated likewise in triplicate and activity determined. Red cell volume was then calculated as follows:

$$\frac{\text{Total radioactivity of blood injected}}{(\text{Activity per 100 ml. of 15 min. cells}) \text{ minus } (\text{activity of initial cells})} = \text{R.B.C. Volume}$$

Following a determination the animal was bled sufficiently to lower the hematocrit to the desired level and after allowing a few days for circulatory adjustment, another determination was made.

The red cell volumes as determined were then plotted against the corresponding jugular hematocrits and a straight line determined by the method of least squares was then drawn.

**EXPERIMENTAL OBSERVATIONS.** Although there is no loss of tagged red cells from the circulation by processes of elimination comparable to the loss of carbon monoxide or plasma volume dyes, nevertheless certain factors operate to require some caution in the analysis of data obtained by this method. If donor cells are injected into a normal dog, the usual circulatory equilibrium is altered and there follows a tendency toward reestablishment of the former status. The result is that blood samples taken a day or so following the injection may show an increase in concentration of radioactivity in the red cells. This is due presumably to the fact that the animal has destroyed some of its own red cells. The cells from the donor animal are typical of red cells in an animal having had hypochromic anemia (by the nature of the preparation of the donor animals (6, 7)), as they are somewhat hypochromic and microcytic in nature, and are therefore somewhat more resistant than are the normal cells of the recipient animal.

We have also encountered another difficulty in the use of this procedure. It has not infrequently happened that the recipient dogs become "sensitized"

to the blood of the donor after repeated determinations are carried out. The symptoms are characteristic of a reaction of transfusion in dogs. There is marked discomfort, a fall in blood pressure, occasionally vomiting, a rapid thready pulse, running reflexes, and usually some spasticity of the hind legs. None of the reactions ended fatally in this series, but some animals were obviously very near death in several cases. Recovery is fairly rapid, usually requiring only about twenty minutes. Blood samples taken from such animals showed extremely marked hemolysis on centrifugation. All of the new isotopic cells had usually disappeared from the circulating cells within ten minutes of the time of injection of the donor cells. It is obviously impossible to determine the red cell volume of these dogs under these conditions, but the difficulty may be circumvented by use of another donor animal.

DISCUSSION. Inspection of figure 1, charts A, B and C, indicates that there is a direct relationship, as is to be expected, between hematocrit and red blood cell mass in the sense that the increased cell mass is accompanied by increased

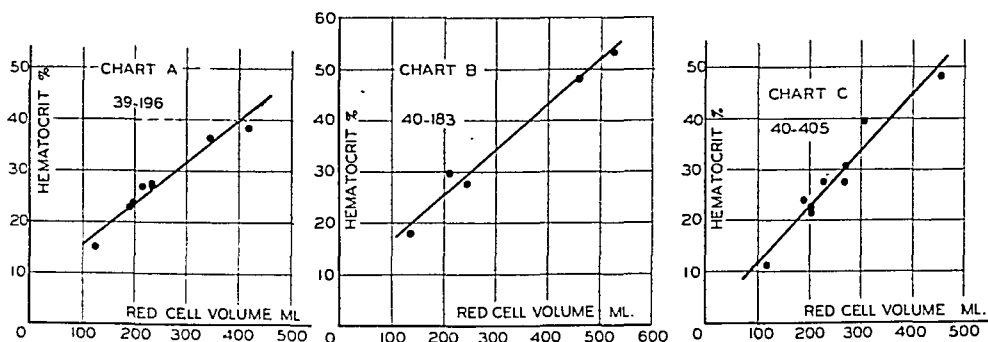


Fig. 1

hematocrit readings. As stated previously, the simplest formulation in this relationship would be:

$$\frac{\text{Hematocrit}}{\text{Red cell mass}} = \text{a constant}$$

Graphically this means that the experimental points should fall on a straight line passing through the origin. In dog 40-405, represented in chart C, the line obtained by the method of least squares is essentially of this character. In charts A and B, however, the straight line of best fit would indicate hematocrit values of 8 for zero cell mass. One suspects, therefore, that these points below a hematocrit of 10 should be fitted to a curve, rather than to a straight line. However, this region of the curve is not pertinent physiologically, since a dog cannot live with a hematocrit of much less than 10.

One may inquire as to the magnitude of the error resulting from the assumption that the red cell mass is proportional to the hematocrit. In chart B, assume that the circulating mass of red cells is known as 480 grams at a hematocrit of 50. By direct proportion at a hematocrit of 25 the red cell mass would be 240. Actually, as determined from the experimental curve, it is 200. The calculated value is thus in error by 20 per cent.

Elsewhere (3) it was shown that the total *circulating* blood volume remains constant with wide changes in venous hematocrit. Data presented here indicate that the cell volume is at least a linear function of hematocrit in the animals investigated. One would expect, therefore, that the circulating plasma value would be a decreasing function of the jugular hematocrit. Actually when one plots venous hematocrit against plasma volume, as determined by the Brilliant Vital Red dye dilution method (9), no close relationship can be demonstrated. This would suggest either that the plasma volume measured by this procedure is unreliable, or that the circulating plasma volume is not the same as the total plasma volume. We have suggested the latter in a recent communication (7). It is possible that the rapidly circulating plasma volume fraction would be inversely proportional to the hematocrit; but in order to prove this it will be necessary to determine this fraction independently of the red cell volume and not by simple calculation based on any assumption of a constancy between the jugular and average body hematocrit (6, 7). Perhaps this may be done by reinspection of the early portions of the curves of optical density of dye in the blood stream plotted against time. If the mixing time of the red cells is only slightly over two minutes (7) in the dog, it is quite possible that the mixing time of the dye in the rapidly circulating plasma fraction is of a similar order. There is no reason to think, in the absence of better evidence, that the rapidly circulating plasma volume is related in any consistent manner to the total plasma volume. Rather it is likely that the amount of plasma in either fraction is dependent on the state of the vascular system at any particular time and the proportion in each may well vary from one site to another simultaneously.

#### SUMMARY

By use of the donor-tagged cell method for the determination of red cell volume in dogs, it has been shown that in the individual dog a linear relationship exists between the red cell volume and the jugular hematocrit over a range of hematocrit of from 11 to 57 per cent.

It is suggested that the total plasma volume as determined by commonly employed methods is not necessarily representative of the actively circulating plasma volume.

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# IDIOVENTRICULAR RHYTHMS AND FIBRILLATION INDUCED AT THE ANODE OR THE CATHODE BY DIRECT CURRENTS OF LONG DURATION

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The brief passage of direct current through the ventricle of the dog via non-polarizable electrodes has long been known to leave the tissues near the electrodes in a charged or polarized state. The discharging occurs gradually over a period of several seconds, rapidly at first and then more slowly, describing an exponential decay curve. Evidences have recently been presented which showed that the delayed responses to brief D.C. stimuli (*ca* 20 msec. duration) applied during the last half of local electrical systole were probably due to a persisting stimulus furnished by the charged state and the consequent polarization current. The response begins at the end of systole and consists of single or multiple premature systoles; sometimes a train of premature systoles leading into fibrillation (15). In order to learn more precisely the effects of weak prolonged constant currents on ventricular activity, and to further analyze the process of the initiation of fibrillation, experiments were designed in which polarity and rate of flow were known and varied at will, while the electrical activities of various areas of ventricular muscle were recorded.

**PROCEDURES.** *Animal preparations.* Twelve dogs, five cats and two monkeys were utilized in these experiments. The results reported are taken mainly from the experiments on dogs which are illustrative of the whole group. Dogs were anesthetized with morphine and sodium barbital, cats and monkeys with dial. The chests of the animals were opened by mid-sternal longitudinal incisions, and the hearts were suspended in pericardial cradles.

*Recording and stimulating methods.* Contiguous riding electrodes (10) led to three large Hindle string galvanometers which were arranged to record on the same 12 cm. paper. In all experiments records were made simultaneously from three local areas on the ventricle while a given test current was applied. The placement of the leads remained constant during all of the trials leading up to one fibrillation, but was varied from fibrillation to fibrillation to record the effects over the various parts of the ventricular surface with a maximum of thoroughness.

The apparatus for the application of the stimulating or polarizing current is shown diagrammatically in figure 1. The 110 volt direct current line, D.C., was connected across a voltage divider, V, which was used to regulate the voltage applied. In the circuit with the tissues was a 10,000 ohm series resistor,  $R_1$ , a milliammeter, A, with a scale reading 0 to 10, and a pole reversing switch,  $S_2$ . Contact with the tissues was made through Ag-AgCl electrodes, a large indifferent one under the skin of the upper end of the chest or of the forelimb, and a stigmatic one on the ventricle. The latter was about 1 mm. in diameter with

the contact end rounded to a bullet nose shape. This cardiac contact electrode was attached to a resilient multi-stranded copper wire in order that it might ride the ventricle, in the manner of the recording electrodes, without exerting undue pressure. The resistance of the tissue and electrode part of the circuit was approximately 1000 ohms. A calibrating circuit with a 1000 ohm resistor,  $R_2$ , was arranged so that by turning a selector switch,  $S_1$ , it could be substituted for the tissue circuit while the current was being adjusted before each test.

The current was allowed to flow for a maximum period of five seconds during each test. It was found experimentally that if fibrillation was to be produced by a current of given strength and polarity it almost always appeared during the first five seconds, usually the first three. The range of current intensities which were found useful was from about 0.5 to 5.0 milliamperes. At each current value records were made first with the cardiac electrode as the cathode, and two minutes later with this electrode as the anode. Neither the sequence of application nor the interval was observed to change the result. However,

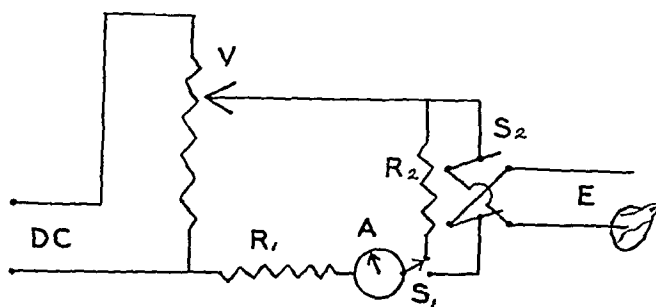


Fig. 1. Apparatus for applying direct current to the ventricle: DC, 110 volt source; V, 193 ohm voltage divider;  $R_1$ , 10,000 ohm resistor;  $R_2$  1000 ohm resistor;  $S_1$ , selector switch;  $S_2$ , reversing switch; A, 0-10 milliammeter; E, Ag-AgCl electrodes.

the arrangement for the cathodal trial to come first, at each current strength, was based upon a good reason which will appear later.

**RESULTS.** Examination of the beats left of the mark (x) in figure 2, A, B, C and D, shows the forms of electrograms recorded by contiguous electrodes during normally initiated beats. The record of a cycle consists essentially of a sharp initial spike and a T wave with an isoelectric line occupying the rest of the time, both systolic and diastolic. Polarizing currents of different strengths and directions cause changes of several kinds. For descriptive purposes we shall consider separately the effects of weak currents which cause no local discharges from the region of the electrode (subthreshold for premature beats), and of stronger currents which do induce local discharges (threshold or suprathreshold for premature beats).

The effects of *subthreshold currents* are illustrated in figure 2, A and B. The current was 2 ma. in both trials. The ventricular electrode (S on heart diagrams) was the cathode in A, and the anode in B. In these records, as in all others with subthreshold currents, the spikes show very little, if any, change in sharpness, width or direction. Spike height is also unchanged here, though

occasionally small changes may be seen. Large changes are evident, however, in the portions between spikes which were isoelectric in the absence of the polarizing current. Leads 1, 2 and 3 (see heart diagram) are recorded in order from

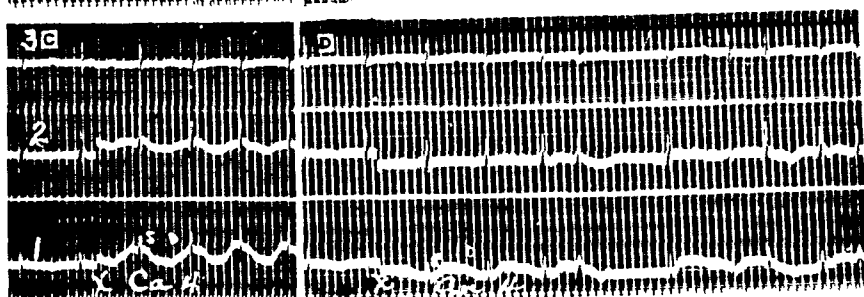
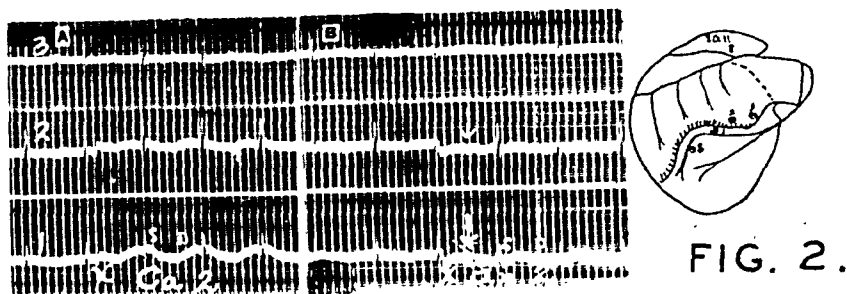


Fig. 2. Normal electrograms from contiguous electrodes and some effects of direct currents. Electrograms from points 1, 2 and 3 on heart diagram are recorded in order from bottom to top in all sections of figure. X, "on" shift of baseline when current was applied; S, moment of contraction of ventricle and D, relaxation; arrows, small oscillations (see fig. 3); vertical lines 40 msec. intervals in all records.

Fig. 3. A, Oscillations near anode; B, near cathode. In A, period is about 80 msec., in B it is 40 msec.

the bottom of the figure upward. In all leads there are "on" and "off" shifts of the base line when the circuit is closed (x) and opened (not shown). The magnitude of the shifts is influenced by the distance of the leads from the

polarizing electrode, their placement with respect to the lines of maximal current flow and the orientation of the two lead points. They are too small to be visible in L-3 of records A and B. In addition to the "on" and "off" shifts, two other changes are seen during the current flow: I. Inflections associated with the phases of muscular activity during the cardiac cycle (*S* and *D*), and II, oscillatory phenomena not temporally correlated with the muscular events (arrows).

The inflections associated with muscular activities are seen in leads 1 and 2. Here it is evident that in every case the systolic inflection (*S*) is in the direction of reducing the base line shift caused by the applied current, and, at the end of systole comes another inflection (*D*) returning the line to the position of maximum shift. In trying to explain these observations experiments were directed toward the detection and evaluation of artifacts which might be responsible for the deflections. The rocking of lead electrodes within the limits seen on a beating heart had repeatedly been shown in the absence of polarization to be without effect on the record. With polarization it was found that a degree of inclination of the electrode with respect to the cardiac surface very much greater than that seen in normal beats was required in order to produce a change in the recorded deflections, and then the most noticeable change was in the height of the spikes, indicating that at extreme inclination the contact was somewhat interfered with. Then the electrodes were supported so that they would only slightly indent the ventricle when relaxed. This made no differential changes in the systolic-diastolic deflections. Supporting the electrode to the extent of reducing the firmness of contact somewhat diminished all deflections including the spikes, the changes thus differing essentially from the phenomena under discussion. Holding the polarizing electrode so that it could not indent the relaxed ventricle was without observable effect. Some tests were made both with and without the 10,000 ohm current stabilizing resistor in the polarizing circuit, the necessary adjustments in voltage being made to keep the current at the desired values. The systolic-diastolic variations were very nearly equal with and without the resistor. This effectively rules out large cyclic changes in current flow as a cause of the deflections since the entire resistance of the electrodes and tissues is only one-tenth as great as the value of the constant resistor. With this resistor in the circuit large changes in tissue resistance could have only a relatively minor effect on the total resistance of the tissue circuit and, therefore, on the current flow.

The most probable explanation is that the systolic-diastolic variations in the recorded effect on baseline level of a constant current are due to changes in conductance in ventricular muscle, as distinguished from total conductance changes in all of the tissues and fluids contained in the circuit. Assuming that the galvanometer records represent potential differences between the two contiguous lead contacts, the direction of the changes is in agreement with the findings of Rapport and Ray (17) on conductance changes in the turtle heart. An increased conductance during systole, current flow being constant, would result in a lower potential difference between the two leads, and the converse

changes would occur during diastole. Suitable rotation of the lead electrodes minimizes base line shift and these deflections. The coincidence of these changes with contraction and relaxation rather than with excitation probably means that the changes in conductance are due to gross physical changes in the muscle and tissue fluids in the region of the pair of leads, not to cell membrane changes of the kind associated with excitation.

In addition to such systolic and diastolic deflections, record 2B exhibits rhythmic potentials with a period of about 40 msec., designated by arrows. Here they are small and require close inspection to be seen. In other arrangements of leads the phenomenon of oscillating potentials in the locality of the polarizing electrode was displayed with greater magnitude and clarity. Figure 3 shows them well. Here lead 1 was only about 7 mm. from the polarizing electrode and the three leads were aligned in the path of probable greatest current density, since the indifferent electrode was in the tissues near the neck end of the thoracic opening. The oscillations have a fundamental period of approximately 40 msec., or a frequency of 25 cycles per second. In many observations from dog and monkey hearts the intervals between crests have always been approximately multiples of 40 msec., i.e., 40, 80 and once 120 msec. In figure 3A the intervals are near 80 msec. with the anodal current of 3 ma., while in 3B the intervals are very close to 40 msec. with a cathodal current of 4 ma. When 4 ma. were applied anodally shortly thereafter there were mixed 40 and 80 msec. oscillations followed by fibrillation. Transitional periods were not seen. It appears reasonable to believe that these oscillations are the cardiac counterpart of the oscillating potentials seen in nerve placed in an environment low in Ca ions and cathodally polarized (1), or upon the mere removal of calcium (5). Oscillating potentials in nerve upon anodal polarization have not been reported, nor have frequencies forming a series of multiples been seen in nerve studies, but fundamentally the processes are probably similar.

*Suprathreshold currents.* When the intensity of the current is above the threshold for idioventricular discharges other changes become evident. Tachycardias and rhythms characteristic of each polarity of current occur. Figures 2C and D are samples. The deflections (*S*) and (*D*) between spikes remain qualitatively similar to those seen with weaker currents but accentuated. The anodal polarization threshold for local idioventricular discharges is about one and a half to two times as high as the cathodal. For example, in this experiment (fig. 2) the anodal thresholds were about 3.5 ma. and the cathodal 2.0 ma. A typical contrast in rhythms is shown in this figure. The cathodal record 2C shows a regular rate, but accelerated over that of the normally initiated beats by about 23 per cent. With stronger currents the increase may be as much as 40 per cent, the rhythm remaining regular. The anodal record 2D shows a coupled type of arrhythmia. The second discharge of each couple originates near the electrode and occurs within the T wave of the previous cycle whose excitation is of normal origin. This is one of the two most frequent types of arrhythmia seen upon anodal stimulation. The other type is shown in figure 4B and C. In this case there is an acceleration of beats until the interval

separating two spikes is of the duration of a normal electrical systole, i.e., a spike falls in the T wave of the preceding cycle. Then one of two things happens: 1. There is a pause ( $X, X$ ) after which begins another series of beats accelerating to the same limit as before, or (2), the accelerating paroxysm quickens its rate of acceleration and eventuates in fibrillation. Both of these sequences of events are seen in figure 4. After coupling also, a short accelerating paroxysm may occur and lead to fibrillation. Exceptionally, coupling and other arrhythmias are seen with cathodal stimulation. These rare responses seem to be more likely to occur in the cat experiments, though the cat responses ordinarily follow the patterns described.

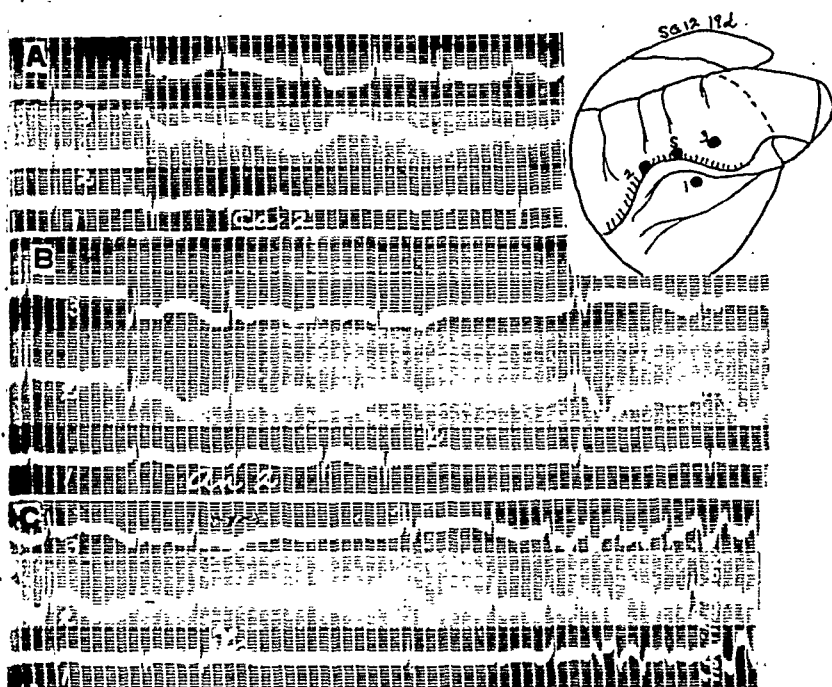
Another feature often seen upon cathodal stimulation is a shifting in precedences of spikes recorded from two locations on the ventricle. The changes in time relations may be of relatively great magnitude. In figure 5A the spikes in leads 2 and 3 (middle and upper electrograms, respectively) show an alternating relationship. In the first beat after the current was applied lead 2 preceded lead 3 by 21 msec., then 3 preceded 2 by 36 msec., 2 led by 21 msec., 3 led by 24 msec., and similarly until the current was stopped. Upon looking at the spikes in only one of these electrograms the impression would be gained that long and short cardiac cycles were alternating, but the intervals are fairly constant between a given spike in lead 2 and the one in the next cycle in lead 3, from this to the next in lead 2, and to the next in lead 3, etc. These relationships suggest that the point of origin of the excitatory discharges is shifting. In the zone about the pole ( $S$ ), the region of highest excitability probably is a ring around the electrode, and the discharge appears to originate in different parts of this peripolar zone, one time nearer lead 3 and next nearer lead 2. Distance, per se, may not be the only factor determining the relative times of excitation at these two points, but unequal accessibility of the two points because of changes in conduction may be a factor. The shifting origin of excitation is further attested by the alternating contours of the spikes in lead 1, indicating a different approach to this area every second beat.

Anodal polarization did not produce comparable shifting relationships between areas. In figure 5B the ventricular excitation is from the normal impulses, the 3 ma. current being subthreshold after the polarity was changed. In figure 4A, however, the cathodal lead 1 follows lead 2 by an interval that varies from 10 to 30 msec., while in the anodal records, B and C, 1 follows 2 by an interval which is much more constant, ranging from 17 to 21 msec.

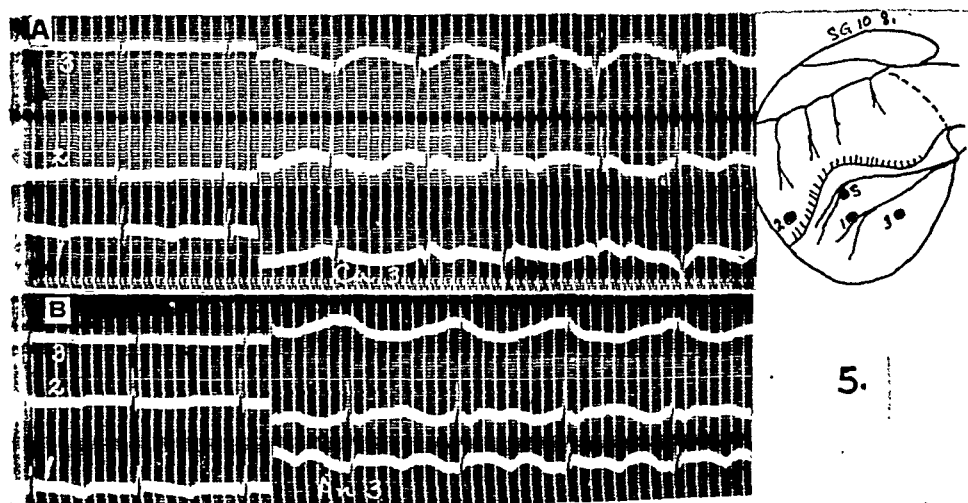
*Fibrillation.* With constant current stimulation fibrillation is primarily an anodally induced phenomenon. A tabulation of all fibrillations in eleven consecutive animals showed 125 fibrillations resulting from anodal stimulation and only 5 from cathodal stimulation. More than 96 per cent of the fibrillations, therefore, were of anodal origin. In many animals 100 per cent of the fibrillations were anodal, and this is the finding to be expected when the current is carefully graded to be kept near the fibrillation threshold, though a very rare cathodal fibrillation will occur at relatively low threshold.

Fibrillation threshold must not be confused with the threshold for excitation

of local systoles. The two, however, are near the same in terms of current readings when anodal stimulation is used. Whenever local irregular discharges



4.



5.

Fig. 4. Characteristic rhythms and fibrillation. A, 2 ma., cathode on ventricle. Local rhythm accelerated over normal rate, but with about normal regularity. B and C, 2 ma. anode on ventricle. Accelerating groups of beats interrupted by pauses at X and X followed by rapidly accelerating series eventuating in fibrillation.

Fig. 5. A, shifts in timing and sequence of spikes at points 2 and 3 with 3 ma. cathodal. Note changing shapes of spikes in L-1 (see text). B, 3 ma. anodal, subthreshold for local rhythms in this case.

or brief paroxysms of beats occur during anodal stimulation the probability is very great that fibrillation will occur within this application of three to five

seconds, or upon repetition of the same current a few minutes later. An increase of a few tenths of a milliampere may be required. With cathodal stimulation locally initiated beats and tachycardias offer practically no prospect of fibrillation. If, for example, local rhythms are induced by a current of 2 ma., fibrillation seldom results from a current of 5 ma., and cannot be depended upon to occur every time at 7 ma., though fibrillation usually will occur with such excessive cathodal currents.

The events leading to fibrillation with an anodal stimulus are shown in figure 6. Fibrillation is introduced by a brief series of premature beats, shown by the spike sequences to be originating in the region of the electrode. The intervals between these beats progressively diminish until the limiting interval of 80 to 90 msec. is reached and then fibrillation supervenes. At lead 1 fibrillation occurs after six premature beats, in lead two after seven or eight, and in

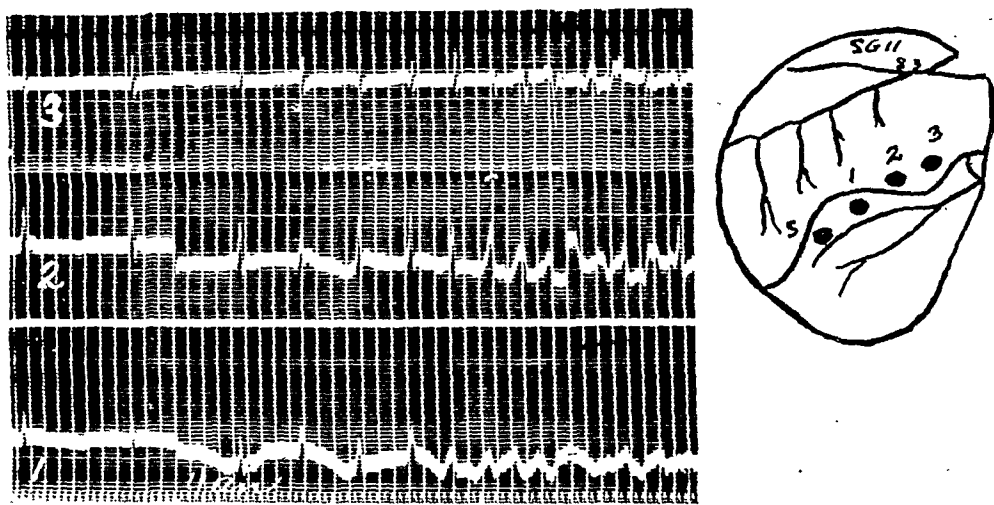


Fig. 6. Anodal fibrillation illustrating induction by discharges originating in region of polarizing electrode at accelerating rate.

lead 3 after eight or perhaps nine. Thus it is seen that the premature systoles and the fibrillation begin in the tissues adjacent to the electrode. The evidences which show that these premature deflections are due to discrete discharges and that they originate at the site of the electrode are the same as those described previously (15) found when fibrillation was evoked by a brief single stimulus placed in the latter part of systole. The train of discharges that introduces fibrillation upon anodal polarization resembles very closely the series that introduces fibrillation after a single brief stimulus. Fibrillation from a moderate (4 ma.) cathodal current occurs so seldom that one cannot say that any form is typical, but the cathodal series of discharges approaching fibrillation has been found to differ from the anodal approach in two ways: 1. The cathodal series of rapid locally induced beats is long, containing about 20 spikes instead of 5 to 8. Such a long rapid bombardment has never been seen to precede an anodal fibrillation. 2. Though there is an accelerating trend in this cathodal



series of rapid beats, there is some slight waxing and waning in frequency as the general overall acceleration takes place.

*Summary of relationships between the effects near the anode and near the cathode when galvanic currents are applied to the mammalian ventricle*

EFFECT	POLARITY	
	Anodal	Cathodal
Production of systolic-diastolic shifts in base line.....	No difference except opposite	that directions are
Production of oscillating potentials.....	Lower waves, longer periods; (80 msec.) likely	Higher waves. Basic periods of 40 msec. likely
Tachycardia or local discharge threshold.....	Higher by $1\frac{1}{2}$ to 2 times	Lower
Rhythm of tachycardia.....	Characteristically irregular	Usually regular
Relation between times of onset of spikes at given separate points.....	Relatively constant	Shifts in timing and sequence
Probability of fibrillation when local discharges are induced.....	Great	Small

DISCUSSION. *Fibrillation: A result of effects of local repetitive activity.* The elucidation of the mechanism of the initiation of ventricular fibrillation, and the discovery of agents and procedures which facilitate or inhibit its development constitute the objective of the series of electrographic studies of which this one is a part. Moe, Harris and Wiggers (15) recently demonstrated that the fibrillation which results from a brief electrical stimulus applied late in systole is preceded by a train of locally initiated beats occurring at an accelerating rate. The present experiments show that the fibrillation that results from the application of constant current is introduced by a similar accelerating series of discharges from the region of the polarizing electrode. From these and other findings in these two studies it may be deduced that this rapid accelerating activity brings about changes in the functioning of the myocardium which allow the intervention of fibrillation. It has long been known that as the heart rate increases electrical systole shortens, the refractory period shortens, and the rate of conduction diminishes (14). Moe, Harris and Wiggers (15) observed upon the application of induction shocks to mammalian ventricles in a serial manner with progressively diminishing intervals of separation that the refractory periods progressively diminished and conduction was slowed in succeeding systoles. Fibrillation was produced if the rhythm of the applied shocks was made to accurately mimic the rhythm of the discharges leading to fibrillation after a brief stimulus. Slowed conduction coupled with a short refractory period are conditions which, sufficiently developed, permit the occurrence of circuitous reëxcitation or fibrillation.

*Anodal and cathodal polarization.* The salient observation in this study of fibrillation by constant currents is that of the pre-eminence of anodal polarization as a fibrillating agent, and, by contrast, the relative impotence of cathodal polarization. However, in the production of locally initiated impulses and tachycardia weak currents are more effective at the cathode than at the anode. The processes involved in the establishment of fibrillation, therefore, must contain some factor or factors which differ significantly from those necessary for mere local initiation of impulses, though these are a prerequisite to fibrillation.

Changes in properties related to excitation which attend anodal polarization, other than the classical electrotonus phenomena, are the development of recovery through supernormality (4) and slowing of accommodation (19).

*Supernormality and fibrillation.* Ashman and Hafkesbring (2) attributed intermittent groups of discharges in turtle heart muscle, initiated by an induction shock, to recovery through supernormality. Treppe and fatigue with attendant increases and decreases in local excitability were offered in explanation of the observed waxing and waning, respectively, in the frequency of discharge during a group, and the further development of fatigue led to the intervals between groups. In our polarization experiments, however, rhythms which closely resembled those of Ashman and Hafkesbring were never obtained. The idioventricular beats which occurred upon *cathodal* polarization were usually found to be quite regular in rate. An exception is the rare case of fibrillation at the cathode with moderate current. A relatively mild intermittence is evident in the long accelerating train of beats. During *anodal* polarization intermittence often occurred, but not with waxing and waning in frequency of discharge. The usual pattern is acceleration throughout the period. If the period of idioventricular impulses ended without fibrillation the end came when the intervals between discharges became reduced until the R spike of one electrogram reached the T wave of the preceding one. The time relation between the end of one systole and the beginning of the next one in such a series of beats was progressively changing from one beat to the next, the two approximating each other more closely with each succeeding cycle. This systematically and rapidly changing relationship is hardly consistent with the view that the discharges result from recovery through supernormality. Many experiments by Moe (unpublished) in which the cardiac cycle was minutely scanned by testing stimuli failed to reveal evidence of a supernormal period in the ventricles (unpolarized) of open chested dogs. A few trials made by us during the flow of weak polarizing currents were negative also, but on account of the smallness of the number of trials and certain technical inadequacies, these were considered inconclusive.

In favor of the view that supernormality could have occurred is the finding of Hoff and Nahum (12) that supernormal excitation occurs in mammalian ventricles under certain experimental conditions and the demonstration of Blair (4) that in nerve anodal polarization produces the property of recovery through supernormality. The coupling type of arrhythmia occasionally seen may reasonably be accounted for as a manifestation of recovery from the first beat

of each couple, usually of normal origin, through supernormality. The second beat in the couple was of local origin and occurred during the latter part of the T wave or shortly thereafter, corresponding very well with the statement of Hoff and Nahum that supernormality occurs during the U wave. However, coupling is not the kind of response which introduces fibrillation, and the evidence at hand does not indicate that supernormality plays an important rôle in its production.

*Accommodation and fibrillation.* Evidences from nerve physiology reveal that a readiness for spontaneous activity, or repetitive discharging in response to a constant stimulus or a single brief stimulus is regularly accompanied by a low rate of accommodation (13). Erlanger and Blair (8) found that sensory nerve fibers repeat more readily than do motor fibers, and correspondingly, sensory fibers accommodate less rapidly than do motor fibers. They have also observed that anodal polarization enhances or produces repetition in excised frog nerve subjected to a constant stimulus (7). Mammalian nerves with circulation intact respond to slightly super-rheobasic currents with repetitive discharges at the cathode or anode, the greater series being elicitable at the anode (18). Accommodation is slow in circulated nerves (20) (16), and anodal polarization has the effect of reducing accommodation while cathodal polarization increases it (19).

Under conditions of slow accommodation, according to B. Katz (13), a single strong brief stimulus applied to a nerve evokes a multiple response in its fibers. In general, the slower the process of accommodation the longer the period of repetitive discharge from a stimulus of given strength. Likewise, the more intense the stimulus (the higher the "local potential," Hill (11)) accommodation being equal, the longer the period of repetition. If the rate of accommodation is sufficiently slow the "local potential" may remain above the threshold during a period long enough to span many discharges and their refractory periods.

If anodal polarization reduces the rate of accommodation in cardiac muscle as it does in nerve, and if cathodal polarization correspondingly increases accommodation, a reasonable explanation is available for the great effectiveness of anodal polarization as a fibrillating agent and for the relative ineffectiveness of currents at the cathode in the production of fibrillation.

Local responses in tissues which exhibit an autogenous excitability cycle like cardiac muscle may be classed in two categories: first, responses in which there is one discharge only upon each local rise of the excitatory process, and second, multiple discharges from a single rise of excitability. In the first group would be the ventricular responses seen upon cathodal polarization and the anodal responses which are spaced more widely than the duration of a normal electrical systole. The regularity of the discharges and the increase in rate above the normal during the tachycardia from a cathodally polarized zone are indicative of a uniformly elevated excitability suggesting the plateau in the cathodal excitability curves found in heart experiments by Gilson and Peugnet (9) and in nerve experiments by Erlanger and Blair (6). This plateau represents a state of balance between two processes, one enhancing irritability ("local potential")

and the other depressing it (accommodation). This regularity of discharge observed during cathodal tachycardias practically eliminates the possibility that any multiple discharges resulted from any single rise of the local excitatory process during these trials. An absence of multiple responses to a single excitation would be a logical result of rapid accommodation. Sufficiently excessive suprathreshold currents, conceivably, could over-ride the accommodation and produce a group of repetitive discharges from a single local cyclic excitation and thus quickly produce the great acceleration of heart rate required for the induction of fibrillation. Excessive currents did sometimes produce such results at the cathode.

In their study of repetitive responses to polarizing currents, Erlanger and Blair (7) found that threshold curves of nerve fibers at the cathode do not always describe a smooth plateau. Those yielding different types of repetitive responses exhibited correspondingly different threshold curves, i.e., rising, falling or oscillating thresholds. A gradually falling threshold at the cathode on the ventricle could explain the gradual acceleration seen on those rare occasions when such acceleration and eventual fibrillation resulted from moderate cathodal stimulation.

In multiple responses resulting from a persisting excitatory state following a single stimulus, autogenous or applied, each discharge should occur immediately upon the restoration of normal excitability. Moe, Harris and Wiggers produced evidence which indicates that this restoration is complete at the end of the T wave. No reduction in threshold for extrasystoles was demonstrable after this, nor was there further shortening of the latent period of extrasystoles evoked by brief stimuli. Discharges which are due to a persisting local excitatory state, therefore, may be expected to occur at or before the end of the T wave. Those which occur appreciably later must be due to a new stimulus. The characteristic short series of about five to seven discharges occurring at short and diminishing intervals which leads to fibrillation upon anodal polarization abundantly obeys the condition prescribed for multiple discharges from a single stimulus. In every case succeeding spikes in these runs of beats have fallen within an interval shorter than the R-T of a normal cycle, and when the limiting interval between spikes of about 80 msec. is reached it is less than half the duration of the R-T segment of a normal electrogram (170-210 msec.). Each time that fibrillation from anodal polarization occurs the same pattern of accelerating discharges is almost repeated, and this typical pattern again is very similar to that which introduces the fibrillation that results from a single brief stimulus. This essential similarity of patterns of series of beats leading to fibrillation, one excited by a single brief stimulus and the other by the anode of a constant current considered together with the fact that anodal polarization reduces accommodation and therefore conveys the capability of multiple responses to a single excitation present a forcible argument that the anodal series is also a multiple response to a single excitatory process arising within the tissue. The essential condition for facilitating the production of fibrillation appears to be a low rate of accommodation.

Other direct effects at the anode, reported in nerve studies, which may well be factors contributing to the establishment of fibrillation are local slowing of conduction and shortening of the refractory period (3). These changes are qualitatively like those produced in the whole ventricle by a rapid series of discharges, however excited. It might be argued that the direct effects of the current at the anode, slowing conduction and shortening refractoriness, constitute the prime factor in the induction of fibrillation. But the well established observations 1, that each fibrillation is preceded by an accelerating series of local discharges, never occurring without them, and 2, that fibrillation is occasionally produced at the cathode without excessive current may be regarded as evidence that the rapid discharges are of main importance in the production of the changes that permit fibrillation. The direct anodal effects of slowing conduction and shortening refractoriness probably play a contributing rôle, though its importance cannot at present be estimated. The indispensable factor for the production of ventricular fibrillation seems to be a very rapid series of locally excited beats.

#### SUMMARY

A method for applying currents controlled as to polarity and rate of flow to the mammalian ventricle is described. Responses from practically the whole ventricular surface were sampled by recording from three local areas simultaneously and changing the positions of the riding contiguous electrode leads from time to time.

Currents, subthreshold for extrasystoles, could give rise to several kinds of changes in the record: 1, "on" and "off" shifts of the base line upon closing and opening the circuit respectively; 2, inflections and changes of level associated with the phases of muscular activity. The amount of displacement of the base-line was reduced during systole, restored to maximal during diastole. After ruling out contact artifacts these features were attributed to conductivity changes associated with contraction and relaxation.

Both anodal and cathodal polarization give rise to oscillating potentials not associated with the phases of the cardiac cycle. The periods of the oscillations were near 40 and 80 msec. Cathodally produced oscillations are higher and are likely to be of the higher frequency (25 cycles per sec., 40 msec. period).

The idioventricular beat threshold was lower for cathodal polarization than for anodal, but the anodal threshold for fibrillation is the lower. The anode is preëminently effective in producing fibrillation. With equal applications of currents near fibrillation threshold 96 per cent of all fibrillations were of anodal origin.

Cathodal polarization of suitable intensity usually produces a tachycardia of regularly spaced idioventricular beats, while anodal polarization produces characteristically irregular rhythms. The point of origin of the discharges appears to be more constant in anodal polarization. In all cases of fibrillation resulting from polarizing currents induction is via an accelerating series of discharges from the region of the polarizing electrode.

In the discussion, findings from studies on excitation and spontaneous rhythmicity in nerve and muscle are considered and correlated with the observations on fibrillation. Absence of, or a low rate of accommodation appears to be the condition essential to spontaneous rhythmicity and multiple discharges in response to a single excitation. The probable association of the reduction of accommodation by anodal polarization with the great effectiveness of the anode in the production of fibrillation is pointed out. Direct anodal effects of locally reducing the conduction rate and reducing the refractory period are considered and assigned a contributing rôle.

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# THE ACTION OF IONS ON THE MAMMALIAN HEART

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There have been few studies of the effects produced on the heart by varying the concentration of Ca, K, Na and H ions within limits close to normal. This is especially true of the mammalian heart. The present study was made to obtain further information concerning the effect on various mammalian heart preparations of varying the concentration of the plasma ions within these limits.

EXPERIMENTAL. Three different types of preparations were used in these experiments. The right atrium preparation of the guinea pig (Spealman, 1940) was used to study the effects of the ions on the rate of beat, the amplitude of contraction, and the duration of contraction of the heart. The term "duration of contraction" is used to mean the total period during which the heart muscle is under increased tension. In these experiments, the procedure was to perfuse the heart with normal Locke's solution for a period of from 20 to 40 minutes; then the experimental Locke's solution which was to be studied was perfused for a similar length of time; and finally, normal Locke's solution was again perfused.

Values for the heart rate, the amplitude of contraction, or the duration of contraction as the case might be, were recorded every 10 or 15 minutes. The heart rate was determined, using a stop watch. The amplitude of contraction was determined by noting the extent of excursion of the heart lever on a millimeter ruler held close to the lever but not touching it. The duration of contraction was determined from kymographic tracings of the heart lever.

The Langendorff preparation was used for electrocardiographic studies on the excised guinea-pig heart. Just as in the experiments with the right atrium preparation, records were taken with the heart perfused with normal Locke's solution both before and after the observations with the experimental Locke's solution. The electrodes used for contact with the heart were silver wire coated with silver chloride. Contact was made by inserting one electrode through the atrial tissue and the other through the ventricular tissue. Only the effects of varying the Ca and K ion concentrations were studied in these experiments.

Dogs anesthetized with Dial or Nembutal were used for observations of the effect of Ca and K ions on the heart in the intact animal. The procedure was to infuse isotonic concentrations of these salts intravenously at the rate of 1 to 3 cc. per minute over a period of an hour or more in order to increase the concentration of either of these salts. Experimental lowering of the serum Ca was accomplished by infusing isotonic sodium oxalate at the rate of 1 to 2 cc. per minute. Lowering of the serum K was brought about by a short (10 min.) intravenous infusion of 1 in 20,000 epinephrine at a rate of 1 cc. per minute. As D'Silva (1934) and others have shown, this causes a rather prolonged lowering of the serum K; so it was possible in the present experiments to allow the

effect of epinephrine to disappear before taking observations. In most of these experiments (see tables 3 and 4) the vagi were cut; for evidence in the literature indicates that the vagus center might be strongly stimulated by infusing  $\text{CaCl}_2$  (Hoff et al., 1939) and possibly by KCl. Electrocardiographic observations

TABLE 1

*The rate of beat, the amplitude of contraction, and the duration of contraction of the guinea-pig right-atrium when perfused with Locke's solution of various compositions*

In each experiment the average of the values obtained with normal Locke's solution was taken as 1.00. The results of several experiments are averaged to give the values in the table. The letters P.D. signify that a progressive decrease in heart rate or in amplitude occurred which prevents the calculation of a numerical value in these cases. — indicates that no data were obtained at that concentration.

## a. Ca ion concentration of Locke's solution varied

	Ca ION CONCENTRATION (M/L)					NUMBER OF HEARTS
	0.0005	0.001	0.002	0.004	0.008	
Rate.....	0.86.	0.98	1.00	1.02	P.D.	3
Amplitude.....	0.12	0.46	1.00	1.50	P.D.	3
Duration.....	—	0.97	1.00	1.17	—	2

## b. K ion concentration of Locke's solution varied

	K ION CONCENTRATION (M/L)				NUMBER OF HEARTS
	0.001	0.002	0.004	0.008	
Rate.....	P.D.	0.98	1.00	0.85	2
Amplitude.....	0.77	0.97	1.00	0.53	2
Duration.....	—	1.22	1.00	1.02	4

## c. Na ion concentration of Locke's solution varied

	Na ION CONCENTRATION (M/L)					NUMBER OF HEARTS
	0.10	0.14	0.15	0.16	0.20	
Rate.....	1.00	0.97	1.00	0.99	0.88	2
Amplitude.....	0.64	1.01	1.00	0.97	0.29	2

## d. H ion concentration of Locke's solution varied

	H ION CONCENTRATION (pH)					NUMBER OF HEARTS
	7.0	7.4	7.5	7.6	7.7	
Amplitude.....	1.01	1.02	1.00	—	1.05	2
Duration.....	—	1.04	1.00	0.98	—	2

(lead II), observations of the intraventricular pressure with the Hamilton manometer, and blood samples were taken at approximately half-hourly intervals. The sera of the blood samples were analyzed for K by the method of Kramer and Tisdall (1921a) or for Ca by the method of Kramer and Tisdall



(1921b). Since only part of the serum Ca exists as Ca ion, the serum Ca determinations only indicate the changes of Ca ion concentrations which took place in the experiments.

TABLE 2

*The electrocardiogram of the guinea pig heart perfused by the Langendorff method with Locke's solution of different Ca and K concentrations*

In each experiment the average of the values found in normal Locke's solution was taken as 1.00. In certain cases the results of several experiments are averaged to give the values in the table (see under remarks).

a. K ion concentration of Locke's solution varied

K ION M/L	R-R	P-R	Q-T	REMARKS
0.003	1.00	1.11	0.91	Average for 2 hearts—2 to 1 block occurred in one
0.004	1.00	1.00	1.00	Normal Locke's solution
0.006	1.03	0.89	1.04	Average for 4 hearts
0.007	1.18	0.87	1.04	One heart, progressive decrease in rate occurred

b. Ca ion concentration of Locke's solution varied

Ca ION M/L	R-R	P-R	Q-T	REMARKS
0.001	1.20	0.75	1.90	One heart, progressive decrease in rate occurred
0.002	1.05	0.90	1.34	Average for 2 hearts
0.004	1.00	1.00	1.00	Normal Locke's solution
0.006	1.06	1.17	1.05	Average for 2 hearts. Occasional block occurred in both

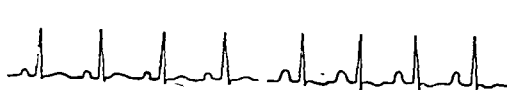


Fig. 1



Fig. 2

Fig. 1. The original electrocardiograms were redrawn as accurately as possible on graph paper to obtain the above reproductions, which illustrate the usual changes occurring in the present experiments during intravenous infusion of  $\text{CaCl}_2$ . In the record to the left (control), the serum Ca was 2.4 mM/L; in the record to the right (taken 105 min. later), the serum Ca was 4.2 mM/L. Note the increase in the P-R interval and in the height of P-wave in record to the right.

Fig. 2. The above reproductions, obtained as in figure 1, illustrate the usual changes in the present experiments occurring during intravenous infusion of KCl. In the record to the left (control), the serum K was 4.3 mM/L; in the other record (taken 85 min. later) the serum K was 6.2 mM/L. Note the decrease in the P-R interval and the increase in the height of the T-wave in the record to the right.

RESULTS. Table 1 summarizes the results obtained on the right atrium of the guinea pig. All values in the table are expressed as decimal fractions of the average of the values obtained with the normal Locke's solution. The table

shows that, within limits close to normal, 1, the heart rate is uninfluenced by variation of the concentrations of Ca, K, or Na ions; 2, the amplitude of contraction increases as the Ca ion concentration increases but is not greatly affected by the other ions studied, and 3, the duration of response is slightly influenced by both Ca and K ions but not by H ion. The table further shows that depressive changes or abnormalities such as a decrease in the heart rate or a depression of the amplitude of contraction, may appear when the concentration of these ions is too different from normal. No depressive changes were found with H ion; this is probably because the concentration was not varied sufficiently.

Table 2 summarizes the electrocardiographic data obtained on the guinea-pig heart perfused by the Langendorff method. All values are expressed as decimal fractions of the average of the values obtained with normal Locke's solution. The data suggest that the R-R interval is affected in these experiments only when the perfusion solution was sufficiently abnormal to cause a lengthening of this interval. The P-R interval decreases as the K ion concentration rises, and increases as the Ca ion rises. The Q-T interval appears not to be greatly



Fig. 3. Intraventricular pressure records illustrating the increase in systolic pressure that usually occurred during intravenous infusion of  $\text{CaCl}_2$ . In the record at the left (control), the serum Ca was 2.4 mM/L; in the middle record (taken 30 min. later), the serum Ca was 4.7 mM/L; and in the record to the right (taken 35 min. after the middle record), the serum Ca was 6.6 mM/L. The pressure values obtained from the above records are respectively 135, 180 and 255 mm Hg. Time 0.2 sec.

affected by K ion concentration, but to be lengthened by a lowering of the Ca ion concentration.

Figures 1, 2 and 3 illustrate some of the positive findings in the electrocardiographic and intracardiac pressure studies in acute experiments with intact animals (dogs). Figure 1 shows that the P-R interval and the height of the P-wave are increased with increasing serum Ca concentration. Figure 2 shows that the P-R interval is decreased and the height of the T-wave is increased with increasing serum K concentration. Figure 3 shows that the intraventricular pressure is increased with increasing serum Ca concentration. A summary of the total findings in these experiments is given in tables 3 and 4. The tables show that the effects illustrated by the figures were present in most of the dogs studied. These tables also give information on other measurements which will be discussed below.

**DISCUSSION.** A difficulty arises in the interpretation of the data obtained in this study. The effect produced when the concentration of any cation of Locke's solution or of the plasma is changed may be a true "physiologic" effect or it may

be an unspecific effect which only signifies that the perfusing fluid is abnormal. This fact must be kept in mind especially when the results in tables 1 and 2 are considered.

In the following discussion, the various activities or properties of the heart are considered under separate headings.

*Heart rate.* The rate of beat of the guinea pig right atrium is not affected by varying the concentration of the Na, K, Ca or H ion, provided these are within limits close to normal. Outside certain limits, the heart rate tends to decrease. This is likewise true of the Langendorff guinea-pig preparation for the ions (Ca

TABLE 3

*Changes in the systolic intraventricular pressure (I.V.P.), in the duration of the intraventricular pressure curve (Dur.), and in the electrocardiogram (lead II) in the intact dog during experimental variation of the plasma Ca concentration*

The number of dogs studied and the average maximum change in the serum Ca are indicated in the first two columns. The table indicates the number of dogs which showed an increase (+), no change (0), or a decrease (−) in the various values at the highest or lowest experimental values reached. Occasionally accurate readings of some of the values could not be obtained; in these cases fewer observations are recorded than there were experimental animals. The vagi were cut in all animals.

NO. OF DOGS	CHANGE IN SERUM Ca, MM/L	HEART RATE			I.V.P.			DUR.			P-R			Q-T			HT. OF P			HT. OF R			HT. OF T		
		+	0	−	+	0	−	+	0	−	+	0	−	+	0	−	+	0	−	+	0	−	+	0	−
5	+2.94	5	0	0	4	1	0	0	0	5	4	0	1	0	1	2	3	0	0	1	0	4	2	1	0
2	−0.90	0	1	1	0	0	2	1	1	0	0	0	2	1	0	0	0	0	2	0	0	2	1	0	1

TABLE 4

*Cardiac changes occurring during experimental variation of the plasma K concentration*

This table is constructed in the same manner as table 3. The vagi were cut in all except one animal in each group.

NO. OF DOGS	CHANGE IN SERUM K, MM/L	HEART RATE			I.V.P.			DUR.			P-R			Q-T			HT. OF P			HT. OF R			HT. OF T		
		+	0	−	+	0	−	+	0	−	+	0	−	+	0	−	+	0	−	+	0	−	+	0	−
6	+1.71	3	2	1	4	2	0	0	0	6	0	0	6	1	1	3	2	3	0	1	3	2	4	2	0
3	−0.90	1	1	1	0	0	3	1	0	2	2	1	0	0	2	1	2	3	0	2	0	0	0	1	2

and K) studied. There is no general agreement in the published literature concerning the physiologic effects of the various ions on the heart rate; and the present findings give no support to the positive findings of others. In the acute experiments on the dog, the heart rate showed a tendency to increase, especially with the infusion of calcium chloride. In this case, the average increase was 25 beats per minute. Others have reported a decrease in heart rate as a result of infusing calcium chloride, supposedly due to vagus stimulation (Hoff et al., 1939); however, in the present experiments the vagi were cut. Possibly, this acceleration in heart rate is due to sympathetic stimulation; however, the heart

rate often remained increased even after infusion was stopped and the serum Ca was approaching its original value.

*Amplitude of contraction.* Ca ion seems to be the chief cation which is believed to affect the amplitude of contraction of the heart. However, there are several reports in the literature indicating that K ion affects the amplitude of contraction in a manner opposite to Ca ion. In the present experiments there was a marked increase in amplitude of contraction of the guinea-pig heart as the Ca ion concentration was increased. Both Na and K ions, when in sufficiently high or low concentration, caused a depression of the amplitude of contraction, which is presumably due to the abnormality of the solutions. In the acute experiments on the dog, variation of the plasma K concentration caused some change in the intraventricular systolic pressure; however, this was small. With increased serum K, there was an average increase in blood pressure of 7 mm. Hg; with decreased serum K, there was an average decrease of 15 mm. Hg. If this slight effect is significant, it should be noted that it is in the opposite direction to that usually postulated for K ion on the excised heart. However, variation of the serum Ca concentration caused marked changes in the intraventricular pressure. The average increase with increased serum Ca was 36 mm. Hg and the average decrease with decreased serum Ca was 65 mm Hg. This effect is in agreement with the fact that Ca ion increases the amplitude of cardiac contraction. It must be admitted, however, that this effect of Ca ion on the intracardiac pressure in the intact animal might not be a direct effect of Ca ion on the heart. A definite conclusion concerning the site of action of Ca ion in these experiments cannot be obtained from the present data.

*Duration of ventricular response.* Since the time of Ringer, it is generally believed that increasing the K ion concentration causes a decrease in the duration of the ventricular response, while increasing the Ca ion concentration increases the duration of the response. The present findings on the guinea-pig heart tend to support the above belief; however, the changes found are of small magnitude. The results obtained on the heart in the dog experiments are difficult to interpret owing to the fact that the heart rate often increased appreciably. This may be the explanation of the slight shortening of the duration of the intracardiac pressure curves in some of the experiments.

*Electrocardiographic changes.* In the following discussion it should be kept in mind that the heart rate and blood pressure showed some variations in the acute experiments with the dog, especially in the case of infusion of calcium chloride. Possibly some of the changes in the electrocardiogram are secondary to these changes.

The studies on the Langendorff preparation of the guinea-pig heart indicate that the P-R interval is decreased by increasing the K ion concentration, and increased by increasing the Ca ion concentration. The experimental findings on the heart in the intact dog are in agreement with those on the perfused guinea pig heart, so far as the P-R interval is concerned. Observations in the literature on the effects of Ca and K ions on the P-R interval are rather few and cannot be discussed here.

Other effects of Ca and K ion on the electrocardiogram were noted. On the excised guinea-pig heart, it was found that the Q-T interval was considerably lengthened when perfused with Locke's solution containing low Ca ion concentrations. This finding is of interest since this alteration of the electrocardiogram has been reported in cases of hypocalcemia in man (Carter and Andrus, 1922). The evidence that the Q-T interval is influenced by the plasma Ca concentration is only suggestive in the case of the present dog experiments.

On the heart of the dog it was further found that the height of the P-wave tended to vary in the same direction as the plasma Ca concentration. The electrocardiographic results obtained on the guinea-pig heart do not show this effect. However, Edwards and Page (1926) found that dogs treated with parathyroid extract sometimes showed an increased P-wave amplitude. The other electrocardiographic results obtained with the intact dog showed no significant variation with the exception that the height of the T-wave showed a tendency to vary in the same direction as the plasma K concentration. This is of interest since an increased amplitude of the T-wave due to increased K concentration has been reported (Nicholson and Schecter, 1937).

#### SUMMARY AND CONCLUSIONS

1. In the guinea-pig right atrium preparation, certain depressive or abnormal changes, such as a decrease in rate which was usually progressive, a depression of the amplitude of contraction, or arrhythmia, occurred when the concentration of the various ions was too different from normal. Within limits close to normal, the most definite positive effect was the variation of the amplitude of contraction with the Ca ion concentration. There was also some suggestion that increasing the Ca ion concentration caused an increase in the duration of the response, while increasing the K ion concentration caused a decrease in the duration of the response.

2. In the Langendorff preparation of the guinea pig heart, the heart rate was independent of the K ion and Ca ion concentrations within regions close to normal, but was depressed in certain instances where the concentration was too different from normal. The P-R interval lengthened as the Ca ion concentration increased, and shortened as the K ion concentration increased. The Q-T interval was lengthened as the Ca ion concentration decreased, but was not greatly affected by changing the K ion concentration.

3. In the acute experiments on dogs, in which the plasma Ca concentration was varied, the intraventricular pressure, the P-R interval, and the height of the P-wave all tended to vary in the same sense with the plasma Ca concentration. The duration of response was slightly decreased and the heart rate was increased when the plasma Ca concentration was raised. The Q-T interval, the height of the R-wave, and the height of the T-wave showed no very definite tendencies to vary with the plasma Ca concentration.

4. In the acute experiments on dogs in which the plasma K concentration was varied, the intraventricular pressure and the height of the T-wave tended to vary in the same sense and the P-R interval in the opposite sense with the plasma

K concentration. The magnitude of the intraventricular pressure changes was small. The duration of response was slightly decreased when the plasma K concentration was raised. The heart rate, the Q-T interval, the height of the P wave, and the height of the R wave showed no definite tendencies to vary with the plasma K concentration.

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# THE INFLUENCE OF BILE SALTS ON ACTIVE INTESTINAL ABSORPTION OF CHLORIDE

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Active intestinal absorption of chloride against concentration gradients has been extensively studied in recent years by Ingraham and Visscher (1, 2, 3), who have shown that under certain experimental conditions the lower ileum can reduce the chloride concentration in the lumen to values as low as 0.5 per cent of the blood plasma level. It appears probable that the active process involved is responsible for a large share of the chloride absorbed by the intestine. In view of the importance of bile salts in the absorption of a wide variety of substances, it seemed desirable to study their influence on active chloride absorption.

**METHODS.** Dogs were anesthetized with pentobarbital sodium injected intraperitoneally. A loop of lower ileum about 2 feet long and about 6 inches from the cecum was isolated. A rubber cannula was inserted in one end of the loop and a bent glass cannula in the other. It was then rinsed out with 3 to 4 liters of isotonic NaCl at about 37° until the washings were clear. A rubber cannula was then substituted for the glass one, and the loop was again rinsed out to remove traces of blood. After the rubber cannulas had been closed with short lengths of glass rod the loop was returned to the abdominal cavity for a few minutes. It was then drawn out and divided into two adjacent loops of equal length by a ligature placed at its center. The loops were returned to the abdominal cavity and allowed to rest for 30 minutes. The dog was kept warm with an electric lamp during the entire experiment.

At the end of the rest period a control solution of half isotonic sodium chloride and half isotonic sodium sulfate at 37° was injected into one loop, and a similar solution containing bile salts was injected into the other. Fifteen minutes were allowed for absorption. The loops were emptied into graduates, and after the volume was read, each was washed out rapidly with 15 cc. of distilled water. The recovered fluid and washings in each case were made up to 50 cc. and analyzed for chloride by the method of Van Slyke (4).

In a large number of experiments the bile salt solutions used had sulfate and chloride concentrations equal or close to those of the controls, but in many cases the distilled water, added to maintain isotonicity, lowered these values considerably. In calculating isotonicity 50 per cent ionization of the bile salts was assumed except in the case of sodium glycocholate in which the calculation was based on the data of Roepke and Mason (5). The sodium chloride present as an impurity in the commercial bile salt, sodium taurocholate, and one of the sodium glycocholate preparations was also considered in estimating isotonicity.

Other experiments were carried out with sodium acetate, glucose, sodium bi-

TABLE 1

*Influence of commercial bile salts on chloride and water absorption*

EXPT.	SOLUTION	ISO- TONICITY	VOLUME		NaCl		Cl ABSORBED
			Original	Final	Original	Final	
			cc.	cc.	per cent	per cent	per cent
1	Control*	1.00	20.0	20.5	0.45	0.40	10
	2 per cent commercial bile salts (CBS)	1.09	20.0	21.2	0.44	0.46	-11
2	Control	1.00	20.0	13.0	0.45	0.24	65
	2 per cent CBS*	1.09	20.0	17.0	0.44	0.45	13
3	Control*	1.00	20.0	13.3	0.45	0.23	67
	1 per cent CBS	1.05	20.0	13.3	0.44	0.36	45
4	Control	1.00	20.0	16.3	0.45	0.36	35
	1 per cent CBS*	1.05	20.0	15.0	0.44	0.44	24
5	Control*	1.00	20.0	14.8	0.45	0.30	51
	0.2 per cent CBS	1.01	20.0	16.0	0.46	0.33	41
6	Control	1.00	20.0	14.0	0.45	0.33	48
	0.2 per cent CBS*	1.01	20.0	14.2	0.46	0.32	51

\* In lower loop.

TABLE 2

*Influence of sodium taurocholate on chloride and water absorption*

EXPT.	SOLUTION	ISO- TONICITY	VOLUME		NaCl		Cl ABSORBED
			Original	Final	Original	Final	
			cc.	cc.	per cent	per cent	per cent
7	Control*	1.00	20.0	16.0	0.45	0.39	30
	2 per cent Na-taurocholate	1.21	20.0	19.5	0.47	0.50	-5
8	Control	1.00	20.0	15.8	0.45	0.27	52
	2 per cent Na-taurocholate*	1.21	20.0	18.5	0.47	0.55	-9
9	Control*	1.00	20.0	14.8	0.45	0.31	49
	1.5 per cent Na-taurocholate	1.00	20.0	16.3	0.41	0.40	20
10	Control	1.00	20.0	15.8	0.45	0.35	39
	1.5 per cent Na-taurocholate*	1.00	20.0	18.0	0.41	0.52	-13
11	Control*	1.00	20.0	15.0	0.45	0.26	56
	1 per cent Na-taurocholate	1.09	20.0	15.0	0.45	0.31	48
12	Control	1.00	20.0	12.0	0.45	0.28	62
	1 per cent Na-taurocholate*	1.09	20.0	12.5	0.45	0.27	63
13	Control*	1.00	20.0	13.0	0.45	0.23	67
	0.5 per cent Na-taurocholate	1.05	20.0	12.7	0.45	0.21	70
14	Control	1.00	20.0	13.3	0.45	0.24	64
	0.5 per cent Na-taurocholate*	1.05	20.0	13.5	0.45	0.29	56

\* In lower loop.



carbonate and saponin. The methods used were identical with those just described.

RESULTS. The experiments with bile salts are described in tables 1 to 4. Two per cent commercial bile salts decreased water and active chloride absorption. The one per cent solution apparently decreased chloride absorption

TABLE 3  
*Influence of sodium glycocholate on chloride and water absorption*

EXPT.	SOLUTION	ISO- TONICITY	VOLUME		NaCl		Cl ABSORBED
			Original	Final	Original	Final	
			cc.	cc.	per cent	per cent	per cent
15	Control*	1.00	20.0	15.8	0.45	0.31	46
	2 per cent Na-glycocholate	1.09	20.0	21.5	0.40	0.56	-50
16	Control	1.00	20.0	14.3	0.45	0.24	62
	2 per cent Na-glycocholate*	1.09	20.0	18.4	0.40	0.49	-13
17	Control*	1.00	20.0	15.0	0.45	0.36	40
	1.5 per cent Na-glycocholate	1.00	20.0	17.3	0.37	0.47	-9
18	Control	1.00	20.0	15.0	0.45	0.36	40
	1.5 per cent Na-glycocholate*	1.00	20.0	17.0	0.37	0.48	-8
19	Control*	1.00	20.0	12.5	0.45	0.20	72
	0.8 per cent Na-glycocholate	1.00	20.0	18.0	0.42	0.52	-13
20	Control	1.00	20.0	17.5	0.45	0.32	39
	0.4 per cent Na-glycocholate*	1.00	20.0	18.0	0.43	0.41	14
21	Control*	1.00	20.0	12.3	0.45	0.24	67
	0.4 per cent Na-glycocholate	1.03	20.0	21.5	0.46	0.40	7
22	Control*	1.00	20.0	16.3	0.45	0.37	32
	0.4 per cent Na-glycocholate	1.05	20.0	17.8	0.48	0.40	26
23	Control	1.00	20.0	13.7	0.45	0.35	47
	0.2 per cent Na-glycocholate*	1.01	20.0	16.3	0.46	0.37	35
24	Control*	1.00	20.0	14.3	0.45	0.25	61
	0.2 per cent Na-glycocholate	1.02	20.0	17.5	0.46	0.32	40

\* In lower loop.

slightly but had no significant effect on water absorption. One and five-tenths per cent sodium taurocholate definitely reduced chloride and water absorption, but concentrations of 1.0 per cent or less had no influence on either process. The absorption of chloride and water was decreased by sodium glycocholate concentrations of 0.2 per cent or higher. Sodium deoxycholate decreased both types of absorption in concentrations as low as 0.2 per cent.

The results obtained with bile salt solutions cannot be explained by reduction

of chloride or sulfate concentrations, for in experiments 36 and 37, table 5, in which sodium acetate or glucose was substituted for bile salts, chloride and sulfate concentrations were considerably reduced with no significant decrease in chloride or water absorption.

TABLE 4  
*Influence of sodium deoxycholate on chloride and water absorption*

EXPT.	SOLUTION	ISO-TONICITY	VOLUME		NaCl		Cl ABSORBED
			Original	Final	Original	Final	
			cc.	cc.	per cent	per cent	per cent
25	Control*	1.00	20.0	13.0	0.45	0.27	61
	2 per cent Na-deoxycholate	1.00	20.0	19.5	0.33	0.47	-39
26	Control	1.00	20.0		0.45		62
	2 per cent Na-deoxycholate*	1.00	20.0		0.33		-80
27	Control*	1.00	20.0	15.5	0.45	0.31	46
	1 per cent Na-deoxycholate	1.00	20.0	21.3	0.39	0.51	-40
28	Control*	1.00	20.0	14.0	0.45	0.28	56
	0.5 per cent Na-deoxycholate	1.00	20.0	17.5	0.42	0.45	5
29	Control	1.00	20.0	20.0	0.45	0.40	11
	0.5 per cent Na-deoxycholate*	1.00	20.0	21.0	0.42	0.55	-38
30	Control*	1.00	20.0	13.3	0.45	0.26	62
	0.3 per cent Na-deoxycholate	1.00	20.0	16.7	0.43	0.49	5
31	Control	1.00	20.0	13.3	0.45	0.32	52
	0.3 per cent Na-deoxycholate*	1.00	20.0	17.0	0.43	0.50	1
32	Control*	1.00	20.0	15.5	0.45	0.32	45
	0.2 per cent Na-deoxycholate	1.00	20.0	18.8	0.44	0.55	-17
33	Control	1.00	20.0	14.3	0.45	0.28	55
	0.2 per cent Na-deoxycholate*	1.00	20.0	16.3	0.44	0.42	23
34	Control*	1.00	20.0	13.5	0.45	0.26	60
	0.1 per cent Na-deoxycholate	1.00	20.0	12.2	0.44	0.27	63
35	Control	1.00	20.0	13.3	0.45	0.30	56
	0.1 per cent Na-deoxycholate*	1.00	20.0	13.8	0.44	0.38	40

\* In lower loop.

Osmotic effects were probably of no importance except in experiments 7 and 8. This seems to be satisfactorily demonstrated by experiments 11 and 12, in which slightly hypertonic solutions produced no appreciable change.

The bile salts, especially sodium glycocholate and sodium deoxycholate, tend to make the solutions alkaline because they are salts of a strong base and weak

acids. The work of Ingraham and Visscher (3) suggests that this may lower the rate of active chloride absorption, but experiments 36, 38 and 39 (table 5) indicate that neither pH nor buffering power can account for the effects of bile salts obtained here. From a consideration of ionization constants (6) and molar concentrations it can be shown that the sodium acetate solution of experiment 36 must have had a higher pH and greater buffering power than the sodium glycocholate solutions having concentrations of 0.8 per cent or less. Yet the sodium acetate solution certainly did not decrease either chloride or water absorption. Similarly it can be shown that in pure water 0.042 per cent sodium bicarbonate has the same pH (9.0) as 0.2 per cent sodium deoxycholate and a higher pH than 0.2 per cent sodium glycocholate. However no significant influence of 0.042 per

TABLE 5

EXPT.	SOLUTION	ISO-TONICITY	VOLUME		NaCl		Cl ABSORBED
			Original	Final	Original	Final	
			cc.	cc.	per cent	per cent	per cent
36	Control*	1.00	20.0	15.0	0.45	0.29	52
	0.19 per cent $\text{NaC}_2\text{H}_3\text{O}_2$	1.00	20.0	13.0	0.39	0.28	52
37	Control	1.00	20.0	14.3	0.45	0.36	43
	0.75 per cent glucose*	1.00	20.0	13.5	0.39	0.34	40
38	Control*	1.00	20.0	13.0	0.45	0.30	57
	0.042 per cent $\text{NaHCO}_3$	1.00	20.0	14.0	0.44	0.27	57
39	Control	1.00	20.0	13.0	0.45	0.29	58
	0.042 per cent $\text{NaHCO}_3$ *	1.00	20.0	13.5	0.44	0.27	59
40	Control*	1.00	20.0	13.0	0.45	0.26	62
	0.3 per cent saponin	1.0	20.0	17.0	0.45	0.42	20
41	Control	1.00	20.0	15.3	0.45	0.32	46
	0.15 per cent saponin*	1.0	20.0	18.0	0.45	0.47	6

\* In lower loop.

cent sodium bicarbonate in an isotonic chloride-sulfate solution was demonstrated in experiments 38 and 39. Commercial bile salts and sodium taurocholate do not appreciably raise the pH of the control solution.

In two experiments saponin decreased chloride and water absorption (expts. 40 and 41, table 5).

DISCUSSION. It should be understood that the methods used do not measure the absorptive process itself but measure net absorption only. Some of the processes which might be modified by the action of bile salts are: secretion or diffusion of chloride, secretion of water, and actual absorption of chloride or water.

In view of the fact that both bile salts and saponin lower surface tension it is possible that their mechanisms of action are similar.

Our knowledge of the bile salt concentrations which can occur in the lower ileum, either normally or in hypermotility, is not yet satisfactory. It is probable that concentrations of the order of magnitude indicated by the present experiments would interfere with salt and water absorption and tend to produce diarrhea.

The author has been unable to find in the literature any reference to effects of bile salts on chloride absorption. It is of interest to note however that Buglia (7) has reported that 0.25 per cent sodium taurocholate decreases water absorption from 0.9 per cent sodium chloride placed in the jejunum in dogs.

#### SUMMARY

The following concentrations of bile salts decreased water and active chloride absorption in anesthetized dogs: 2 per cent commercial bile salts, 1.5 per cent sodium taurocholate, 0.2 per cent sodium glycocholate, and 0.2 per cent sodium deoxycholate.

In general higher concentrations than these produced greater effects. The net transfer of chloride or water was toward the intestinal lumen in some cases.

Nineteen hundredths per cent sodium acetate, 0.75 per cent glucose, and 0.042 per cent sodium bicarbonate did not significantly decrease water or active chloride absorption.

The results obtained with bile salts cannot be accounted for by reduction of original chloride and sulfate concentrations, osmotic effects, pH, or buffering power.

Fifteen hundredths per cent saponin decreased water and active chloride absorption.

The author is indebted to Prof. Lathan A. Crandall, Jr., who suggested this problem, for valuable criticism and advice.

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# THE EFFECT OF LOW POTASSIUM DIET AND OF DESOXYCORTICOSTERONE ACETATE UPON RENAL SIZE<sup>1</sup>

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During the course of experiments on the survival of nephrectomized rats previously fed a diet low in potassium, it was observed that this diet caused renal hypertrophy. Similar changes followed the administration of desoxycorticosterone acetate.<sup>2</sup> The following data are presented to elucidate the nature of these phenomena.

**REVIEW OF LITERATURE.** While studying the effects of potassium deficiency on tumor-bearing mice, Liebow, McFarland and Tennant (1) observed increase in kidney weight associated with tubular dilatation, hypertrophy and epithelial proliferation. They also were able to show that these changes could be reversed upon re-alimentation of the animals. Winters, Smith and Mendel (2) noted a similar increase in renal size of rats fed a salt-free diet. Kidney enlargement in rats occurred following prolonged administration of desoxycorticosterone acetate, according to Selye (3); a finding confirmed by many (4) (5). Similar renal changes have also been produced in rats and mice following injections of steroids of the ovary and testis (6) (7) (8).

**METHODS.** Adult albino, male and female rats, of approximately 200 grams, were used in all experiments. A control group was fed on Purina Dog Chow. The basic ingredients of the low potassium diet were present in the following proportions (9): commercial dextrin (yellow corn), 32 grams; sucrose, 25 grams; vegetable fat (Crisco), 22 grams; commercial lactalbumin (Borden's), 18 grams; dried brewer's yeast (Harris), 2 grams; bone ash, 2 grams; cod liver oil, 1 gram; and sodium chloride, 1 gram. One group of rats was fed the above diet plus 1 gram of KCl (group 3). The potassium content of the stock diet (Purina Dog Chow) was 15.5, m M/100 grams, that of the low potassium diet 1.6 m M/100 gram, and that of the synthetic diet with added potassium chloride 16.6 m M/100 grams. All animals had free access to distilled water with the exception of groups 6 and 7, to whom unlimited quantities of a 1.5 per cent solution of KCl was offered.

Crystalline desoxycorticosterone acetate was dissolved in warm alcohol and added to physiological saline so that each cubic centimeter contained 2 mgm. of finely suspended precipitate and 7 per cent alcohol. Daily subcutaneous injections of 2 mgm. of desoxycorticosterone acetate were given to groups 4, 5 and 6 during a period of four weeks.

<sup>1</sup> Aided by a grant from the Commonwealth Fund.

<sup>2</sup> The crystalline desoxycorticosterone acetate, percorten, used in this study was supplied through the courtesy of the Ciba Pharmaceutical Products, Inc., Summit, N. J.

The animals were sacrificed following etherization and fresh kidney weights determined. To secure dry weights the organs were placed in an oven at 100°C. The latter determination was not made in all instances as it was deemed essential to have fresh material for histological study. In these cases, the dried weights of the two kidneys were assumed to be proportionate to that of the single kidney.

RESULTS. The tabulated results of experiments are presented in table 1.

*Effect of low potassium diet.* Significant increase in the kidney weights of rats fed a potassium deficient diet (group 2) occurred when the results were compared to the control groups fed either a similar diet with added potassium (group 3) or stock diet (group 1). The increase in the dry weights was of the same order of magnitude. Histologically the changes were confined to the mid-medullary zone, where dilatation of the tubules with marked hyperplasia of the lining epithelium occurred, as indicated by columnar cells with numerous mitotic

TABLE 1

*The effect of diet and desoxycorticosterone acetate on kidney size*

GROUP	DIET	INJECTIONS*	DRINKING FLUID	NUMBER OF RATS	MEAN BODY WEIGHT	KIDNEY WEIGHT PER KILO	t†	DRY KIDNEY WEIGHT PER KILO	t†
					grams	grams		grams	
1	Stock	0	H <sub>2</sub> O	10	201	7.62 ± 0.41	0.00	1.77 ± 0.09	0.00
2	Low K	0	H <sub>2</sub> O	6	248	10.53 ± 0.15	16.80	2.01 ± 0.12	3.90
3	Normal K	0	H <sub>2</sub> O	5	257	7.37 ± 0.60	0.96	1.69 ± 0.09	1.43
4	Low K	Doca	H <sub>2</sub> O	4	151	10.83 ± 0.81	10.00	2.43 ± 0.17	9.44
5	Stock	Doca	H <sub>2</sub> O	18	222	9.07 ± 0.68	5.10	2.14 ± 0.14	6.79
6	Stock	Doca	1.5% KCl	5	190	7.37 ± 0.34	1.18	1.75 ± 0.08	0.40
7	Stock	0	1.5% KCl	5	204	7.22 ± 0.44	1.81	1.68 ± 0.38	1.84

\* Doca indicates 2 mgm. of desoxycorticosterone acetate per day for 28 days.

† t according to Fisher (10). Value of t greater than 3.0 indicates significant difference at  $p < 0.01$ .

figures (figs. 1 and 2). The portions of tubule involved included the ascending loop of Henle and the collecting tubules.

*Effect of desoxycorticosterone acetate.* The prolonged administration of large doses of desoxycorticosterone acetate produced enlargement of the kidneys, as evidenced by the increase of both wet and dry weights. These changes were not as marked as those found in rats fed the low potassium diet. Similarly, the histological changes, while of the same nature, were not as pronounced.

*Effect of low potassium diet plus desoxycorticosterone acetate.* The changes in animals on a low potassium diet and injected with large quantities of desoxycorticosterone acetate were similar to those observed in the animals receiving the diet alone, and were only slightly more marked. Histologically, the changes in the two groups could not be differentiated.

*Effect of desoxycorticosterone acetate plus high potassium.* The animals that received large doses of desoxycorticosterone acetate over a long period of time, supplemented by a 1.5 per cent solution of potassium chloride as the only source

of fluid, manifested no increase in renal size or changes in the concentration of water in the kidneys. No gross or microscopic alterations were evident in any of the tissues of these animals.

*Effect of high potassium.* Animals receiving a stock diet (Purina Dog Chow) plus a 1.5 per cent solution of potassium chloride as drinking water showed no changes in kidney size and no gross or histological changes in the tissues.

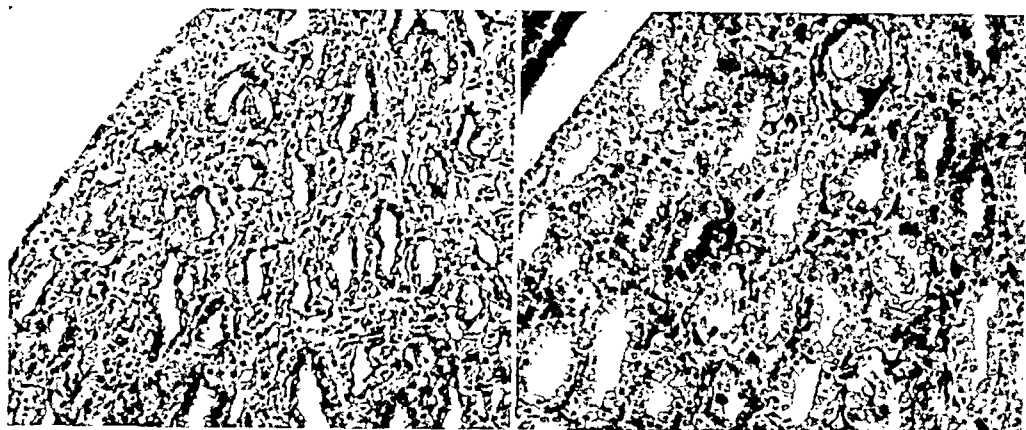


Fig. 1

Fig. 2

Fig. 1. Group 1, stock diet. Pyramid of kidney.  $\times 130$

Fig. 2. Group 2, low K diet. Pyramid of kidney.  $\times 130$

**DISCUSSION.** Enlargement of the kidneys has been demonstrated previously in mice fed a diet low in potassium (1) and in rats receiving injections of desoxycorticosterone acetate. The present paper emphasizes the close relation of the two phenomena. Diets low in potassium lead to loss of muscle potassium and gain in muscle sodium (11) (12). Furthermore, injections of desoxycorticosterone acetate produce similar changes in the muscle (13) (14). Both conditions are accompanied by abnormally low serum potassium. Injections of adrenal cortical substances, as well as steroids of ovary and testis, produce increased urinary excretion of potassium (15) (16). Apparently the kidney is unable to prevent loss of potassium when animals are fed a diet low in potassium or when they receive injections of desoxycorticosterone acetate. It seems likely that the tubular hypertrophy found in these two conditions is connected with an attempt of the renal tubules to reabsorb potassium from glomerular filtrate. In the case of the low potassium diet, realimentation shows that normal ability to reabsorb potassium persists (1); on the other hand desoxycorticosterone acetate interferes with the ability to reabsorb potassium in spite of normal potassium intake. In both cases a similar tubular hypertrophy results.

The rôle of potassium is indicated by the prevention of hypertrophy when KCl supplements the low potassium diet or is added to the drinking water in rats given desoxycorticosterone acetate. These facts indicate that the tubular hypertrophy after injection of desoxycorticosterone acetate should not be regarded as a form of growth produced by hormonal stimulus but rather as hypertrophy associated with the attempt to reabsorb potassium in the presence of a deficiency.

## SUMMARY

Hypertrophy of the kidney results in the rat after four weeks of a low potassium diet. The increased weight of this organ is accompanied by no significant proportionate change in the water content. The anatomical alterations are confined to the loops of Henle and collecting tubules and consist of dilatation, hypertrophy and hyperplasia.

Similar, though less marked, renal changes occur following the administration of desoxycorticosterone acetate to rats on a normal diet. These alterations are prevented by the addition of KCl to the drinking water.

Animals subjected to a high potassium regime alone show no anatomical changes.

Grateful acknowledgment is made for the technical assistance of the Misses Gladys Hammond and Virginia Lane.

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## ION ANTAGONISM AND THE FROG HEART

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The current concept of ion antagonism seems to be somewhat similar to that held by Sydney Ringer, namely, that a given pair of ions oppose each other in their action on all activities and properties of the heart. While it is probably realized that this is not strictly true, there is little experimental information to indicate the extent of this opposing action.

The present report is an attempt to describe the concomitant action of one pair of ions, Ca and K, on the frog heart. Two<sup>1</sup> possible manifestations of antagonism are considered: 1, toxicologic, and 2, physiologic.

The experimental part of the present communication concerns only toxicologic effects. Specifically, the attempt is here made to determine if the physiologic limits of  $\text{Ca}^{++}$  concentration can be modified by changing the  $\text{K}^+$  concentration.

**EXPERIMENTS.** All experiments were carried out on the frog (*Rana pipiens*) heart. The heart was perfused through the sinus venosus in the manner previously used (Spealman, 1938).

The observations made were to determine if a given solution was "toxic". The primary criterion was the heart rate, i.e., the rate of beat of the sinus venosus; for it has been shown (Daly and Clark, 1921; Spealman, 1938) that excessively high or low concentrations of any one kind of ion in an otherwise normal Ringer's solution causes slowing or complete arrest of the heart.

The procedure was to perfuse the heart with the chosen experimental solution after observations of the rate of the heart perfused with the control Ringer's solution had been made. The usual length of time of perfusion was 30 minutes, with observations on the heart recorded every 10 minutes. If the heart rate showed no change, another experimental perfusion fluid was tried; however, if the heart showed an appreciable decrease in rate, it was immediately perfused with the control Ringer's solution to determine if the change had been due to the experimental solution or to some other factor. Often it was not necessary to perfuse an experimental solution as long as 30 minutes to demonstrate that it was toxic; but occasionally it was necessary to perfuse the heart longer in order to

<sup>1</sup> Ringer (1887) divided the possible antagonistic action into these two sub-divisions.

make a decision. Observations on the presence and degree of heart block were also made.

Two series of perfusion fluids were used. In each series the concentration of  $\text{NaHCO}_3$  and of  $\text{NaCl}$  were the same, 2 mM/L and 110 mM/L respectively. In one series the  $\text{KCl}$  concentration was 1 mM/L and in the other, it was 4 mM/L. The  $\text{CaCl}_2$  concentration was varied in each series from 0.125 to 8.00 mM/L. For each series, the solution containing 2.00 mM/L of  $\text{CaCl}_2$  was used as the control solution.

Thus two sets of experimental solutions were employed, one having a  $\text{K}^+$  concentration near the upper limit of normal and one having a  $\text{K}^+$  concentration near the lower limit of normal. For each of these two sets of solutions, the limits

TABLE 1

*Heart rate.* The heart rate in the control Ringer's solution (2.0 mM/L Ca ion) in each case is taken as 1.00. The subscripts indicate the experimenter's estimate of the significance of the decrease in rate, when such factors as spontaneous variation in heart rate, progressive nature of the change, etc. are considered. (d) represents a significant decrease, (n) no significant decrease, — indicates that no reading was taken.

Heart	0.125	0.25	0.50	2.00	4.00	8.00
<i>Calcium ion concentration in mM/L</i>						
a. K ion concentration of all solutions, 1 mM/L						
1	0.62 <sub>d</sub>	1.02 <sub>n</sub>	—	1.00 <sub>n</sub>	1.00 <sub>n</sub>	0.72 <sub>d</sub>
2	0.73 <sub>d</sub>	0.94 <sub>n</sub>	1.00 <sub>n</sub>	1.00 <sub>n</sub>	0.86 <sub>n</sub>	0.60 <sub>d</sub>
3	0.44 <sub>d</sub>	0.89 <sub>n</sub>	—	1.00 <sub>n</sub>	0.91 <sub>n</sub>	0.69 <sub>d</sub>
4	0.95 <sub>n</sub>	1.01 <sub>n</sub>	0.92 <sub>n</sub>	1.00 <sub>n</sub>	—	0.63 <sub>d</sub>
5	0.48 <sub>d</sub>	0.81 <sub>n</sub>	—	1.00 <sub>n</sub>	0.93 <sub>n</sub>	0.38 <sub>d</sub>
b. K ion concentration of all solutions, 4 mM/L						
1	0.55 <sub>d</sub>	0.96 <sub>n</sub>	0.93 <sub>n</sub>	1.00 <sub>n</sub>	0.84 <sub>n</sub>	0.65 <sub>d</sub>
2	0.48 <sub>d</sub>	0.83 <sub>n</sub>	0.75 <sub>n</sub>	1.00 <sub>n</sub>	0.86 <sub>n</sub>	0.65 <sub>d</sub>
3	0.78 <sub>d</sub>	0.85 <sub>n</sub>	0.67 <sub>n</sub>	1.00 <sub>n</sub>	0.95 <sub>n</sub>	0.68 <sub>d</sub>
4	0.49 <sub>d</sub>	0.93 <sub>n</sub>	0.97 <sub>n</sub>	1.00 <sub>n</sub>	0.93 <sub>n</sub>	0.54 <sub>d</sub>
5	—	0.59 <sub>d</sub>	1.13 <sub>n</sub>	1.00 <sub>n</sub>	1.14 <sub>n</sub>	0.77 <sub>n</sub>

of  $\text{Ca}^{++}$  concentration which would allow the heart to continue beating at its normal rate for a reasonable period of time was determined, as explained above.

**RESULTS.** Table 1 gives the data on the behavior of the heart rate in these solutions. The data show that the limits of  $\text{Ca}^{++}$  concentration in which the heart rate is not significantly decreased from the control are apparently the same whether the solutions contained 1.00 or 4.00 mM/L of  $\text{K}^+$ .

Table 2 summarizes the data on the behavior of the heart as regards atrio-ventricular conduction. The data were obtained on the same hearts as the data of table 1. N indicates a normally behaving heart, and B indicates that block occurred. The plus signs after the B's indicate the extent of block (see legend of table). The data indicate that the region of  $\text{Ca}^{++}$  concentration in which heart

block is absent shifts to higher  $\text{Ca}^{++}$  concentrations, as the  $\text{K}^+$  concentration of the solution is increased from 1 to 4 mM/L.

**DISCUSSION.** Sydney Ringer's evidence for a physiologic antagonism between the ions was much stronger than his evidence for a toxicologic one. However, the physiologic antagonism is not so complete as is generally believed. For example, there is no physiologic antagonism between  $\text{Ca}^{++}$  and  $\text{K}^+$  so far as the heart rate is concerned, for neither ion in physiologic concentrations affects the heart rate (Spealman, 1938). Likewise, there appears to be only a very feeble antagonism (Clark, 1926) or none at all between ions so far as the amplitude of contraction is concerned; for, although  $\text{Ca}^{++}$  influences this activity,  $\text{K}^+$ , within

TABLE 2

*Heart block.* N indicates heart block did not occur. B+ indicates an atrio-ventricular block of less than 2 to 1; B++, greater than 2 to 1, but not complete; and B+++ indicates a complete atrio-ventricular block. The B's with plus signs, following some of the N's in the control (2.00 mM/L Ca ion) column, indicate that block was present with this solution during the latter part of the experiment. The asterisk above and to the left of the B indicates the heart block occurred after it appeared in the control solution. — indicates no reading was taken.

Heart	0.125	0.25	0.50	2.00	4.00	8.00
<i>Calcium ion concentration in, mM/L</i>						
a. K ion concentration of all solutions, 1 mM/L						
1	N	N	—	N	B++	B++++
2	B++++	B+	N	N	B+	B++++
3	N	N	—	N	B++	B++++
4	B++	B++	N	N(B++++)	—	B++++
5	B+	N	—	N(B++)	B++++	*B++++
b. K ion concentration of all solutions, 4 mM/L						
1	B+	B++	N	N	N	N
2	B++++	B++	B+	N	N	N
3	B++++	B++	*B++	N(B++)	N	*B++
4	N	N	N	N	N	N
5	—	B++	B+	N	N	N

the physiologic range, has little (Clark, 1926) or no (Spealman, 1940) effect on the amplitude. However, certain properties or activities of the heart are apparently influenced in opposite directions by these ions. For example, Colle (1927) has shown in careful quantitative studies that  $\text{Ca}^{++}$  and  $\text{K}^+$  change the excitability of the frog heart in opposite directions over a wide range of concentration of these ions. Likewise, the duration of the mechanical response appears to be affected in opposite senses by  $\text{Ca}^{++}$  and  $\text{K}^+$  (Ringer, 1887). There are probably other activities and properties of the frog heart which are affected in opposite manners by these ions. It is likely that  $\text{Ca}^{++}$  and  $\text{K}^+$  are mutually antagonistic in these cases. However, it should be emphasized that these two ions are not

mutually antagonistic within physiologic limits of concentration on all activities or properties of the heart.

The antagonism existing between toxic concentrations of these ions shows a similar situation. So far as the rhythmic function of the pacemaker of the heart is concerned (table 1), there appears to be no antagonism between  $\text{Ca}^{++}$  and  $\text{K}^+$ ; for the limits of  $\text{Ca}^{++}$  concentration in which the heart rate is not depressed are uninfluenced by the  $\text{K}^+$  concentration. The results obtained in the studies of heart block do show that antagonism exists between these two ions in this case; for the range of  $\text{Ca}$  concentration in which block is absent shifts to higher values with increase in the  $\text{K}^+$  concentration of the solution.

#### SUMMARY AND CONCLUSIONS

1. The region of  $\text{Ca}^{++}$  concentration in which the pacemaker of the heart is able to maintain its rhythmicity unimpaired does not appear to be influenced by the  $\text{K}^+$  concentration.

2. The region of  $\text{Ca}^{++}$  concentration in which the heart is free from atrio-ventricular block is shifted to higher concentrations as the  $\text{K}^+$  concentration is increased.

3. From the discussion, it is apparent that the antagonism between  $\text{Ca}^{++}$  and  $\text{K}^+$  is of a limited nature in the case of the frog heart.

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# A DEFECT IN THE COAGULATION MECHANISM OF SWINE BLOOD<sup>1</sup>

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About 3 years ago it was discovered that a considerable number of swine in the Missouri Agricultural Experiment Station herd were subject to severe and protracted hemorrhage. The abnormal individuals would sometimes bleed to death from a very slight injury, or no injury at all that could be detected. The bleeders are progeny of an inbreeding investigation and the affected animals are closely related. Hogan et al. (1) reported some preliminary diagnostic observations and concluded that the disease is similar to hemophilia.

The more important characteristics observed up to date may be summarized as follows: 1. The hemorrhagic tendency is inherited; both sexes have the coagulation defect and both transmit it. 2. The bleeding time by Duke's (2) method is normal. If the wound is more extensive, however, the bleeding time is prolonged. 3. The whole blood coagulation time is greatly extended. 4. The clot retracts normally when once formed. 5. There is no deficiency of calcium. 6. Howell's "prothrombin time" (3) is greatly prolonged. 7. Prothrombin is present in normal concentration. 8. The platelets are normal in number but they are abnormally stable. 9. Fibrinogen is present in normal amount. 10. When the blood comes in contact with injured tissues it coagulates in normal time. The blood is deficient in readily available thromboplastin.

The purpose of this paper is to describe: 1, a diagnostic test that is suitable for our purpose; 2, additional features of the bleeding abnormality.

**EXPERIMENTAL.** The routine of swine management, such as marking, putting rings in the nose, and castrating the males, requires the infliction of minor wounds. In addition the habits of the animals expose them to injuries that are ordinarily of no consequence but are extremely hazardous to individuals with the hemorrhagic tendency. The animals most useful for study are the ones most likely to be lost and it was necessary to devise diagnostic tests that would make it possible to protect them.

**Diagnostic tests.** Our early experience with prolonged hemorrhage from apparently insignificant scratches led us to believe that a bleeding time test would be satisfactory. In the spring of 1939, therefore, all of the pigs in the bleeder line were tested by cutting nicks in the edge of the ears. It was supposed that the animals which gave no indication of hemorrhage were normal but later when the boars were castrated eight that had given a normal test bled to death. Out

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of the 23 animals which proved by hemorrhage at one time or another to be bleeders, only five were abnormal by the bleeding time test. Two of these bled to death within an hour after the nicks were made and the other three had narrow escapes but were saved by putting hemostatic clamps on each side of the wound. It is evident then that a test for the disease based upon bleeding time must be made on a relatively large wound if it is to be reliable, but a test of this kind that is reliable is too dangerous to use. The methods that have been seriously considered are described below.

1. *Needle test.* A 19 gauge petrolatum coated needle is inserted in the marginal ear vein. If the blood stops dripping from the external end of the needle because of clot formation in the needle in less than 5 minutes after the appearance of the first drop of blood, the animal is normal. If a clot does not form the animal is a bleeder.

2. *Microscopic test.* Twenty-five minutes after the blood is drawn into an iced paraffined tube, a smear is made on a glass slide, dried, stained by Wright's method (4) and examined under oil immersion. If the platelets are clumped and ragged the animal is normal. If the platelets have sharp outlines and are only grouped the animal is a bleeder.

3. *Whole-blood coagulation time.* The procedure is essentially the same as the Lee-White (5) modification of Howell's method. Five milliliters of blood are collected by venous puncture in cold paraffin coated tubes and placed in an ice bath. It has been found that the coagulation time is less than 40 minutes in a normal animal. If the coagulation time is 50 minutes or more the animal is probably a bleeder.

4. *Fibrin precipitation test.* (6) Four milliliters of blood are drawn by careful venous puncture into a 15 ml. paraffin coated centrifuge tube containing 1 ml. of 3.8 per cent sodium citrate. The tube is stoppered, the contents thoroughly mixed, cooled in a refrigerator at 5°C. for one hour and centrifuged at 2800 r.p.m. for 25 minutes in a centrifuge having a radius of 20 cm. The time of standing and the rate of centrifuging are important. One milliliter of the citrated plasma is mixed with a glass stirring rod in a 50 ml. graduated cylinder with 48 ml. of 0.9 per cent sodium chloride and 1 ml. of 2.5 per cent calcium chloride. If the plasma is normal it will form a mass of fibrin throughout causing the mixture to become turbid in about 16 minutes. The fibrin precipitation process is always complete, when normal plasma is used, in 20 minutes. On rotating the glass rod the fibrin is wrapped around it and the contents of the cylinder are clear again. It requires about 35 minutes for the precipitation of bleeder fibrin under these conditions.

In an effort to determine whether or not the proposed tests would consistently distinguish between normals and bleeders, three normals and three confirmed bleeders were tested by all four methods as shown in table 1.

Table 1 indicates that tests 1 and 4 are the most consistent, presumably because there is less danger of interfering contamination. These two tests were then applied to 74 animals, and 27 were bleeders and 39 were normal by both. In 8 cases, however, the blood continued to flow through the needle but when

tested for fibrin precipitation time the fibrin came down in less than 20 minutes. It developed later on that none of these animals was a bleeder.

Several of the animals that were diagnosed as bleeders had never shown any signs of hemorrhage, but with one exception they all developed external bleeding tendencies later. Boar 28 was the exception but when retested his blood flowed freely and the fibrin precipitation-time was thirty-five minutes. In our experience the fibrin precipitation test is more dependable than any other yet tried. Other advantages are: 1, the use of citrate rather than oxalate for the

TABLE 1  
*Tests for abnormal swine*

NO.	ANIMAL	TEST 1 FLOW THROUGH NEEDLE	TEST 2 WHOLE- BLOOD COAGULA- TION TIME	TEST 3 MICROSCOPIC EXAMINATION OF PLATELETS	TEST 4 FIBRIN PRE- CIPITATION	REMARKS
		<i>min.</i>	<i>min.</i>		<i>min.</i>	
1	Normal	3	41	Much grouping	15	All reliable
2	Normal	1	13	Small groups	18	All reliable
3	Normal	2	57	Small groups	18	Test 2, failed
4	Defective	More than 5	98	Much grouping	Over 20	Test 3, failed
5	Defective	More than 5	85	Platelets single	Over 20	All reliable
6	Defective	More than 5	80	Platelets single	Over 20	All reliable

TABLE 2  
*Reliability of the fibrin precipitation test*

ANIMAL NO.	FIBRIN PRE- CIPITATION TIME	DIAGNOSIS	REMARKS
	<i>min.</i>		
7	11	Normal	From a normal line
8	50	Bleeder	Died from hemorrhage
9	16	Normal	Did not bleed excessively when castrated
10	30	Bleeder	Bled profusely when castrated
11	8.5	Normal	From a normal line
12	48.0	Bleeder	Died from spontaneous internal hemorrhage one week after testing
13	18	Normal	From a normal line
14	36	Bleeder	Had been bleeding from gums for two days
15	16	Normal	From a normal line
16	41	Bleeder	Profuse hemorrhage from an ear injury

anticoagulant leaves a clear solution, in place of a white cloudy suspension, when the plasma is recalcified; 2, the end point is visible, very definite, and can be read without inverting the tube. This increases the accuracy because agitation accelerates the coagulation process; 3, a number of samples can be determined simultaneously; 4, the final readings do not need to be made immediately after securing the sample. After the blood is drawn it can be removed to a more convenient laboratory for completion.

Typical results are shown in table 2.



*Characteristics of the bleeding abnormality.* It seemed that the most useful guide in planning future work would be the experience of workers who had studied a similar abnormality, and our observations have been compared with those made on various blood-coagulation defects. Up to the present time the

TABLE 3  
*Quick's methods for the diagnosis of hemophilia*

TESTS DESCRIBED BY QUICK	QUICK'S VALUE FOR NORMAL MAN	AVERAGE VALUE FOR 5 NORMAL SWINE	QUICK'S VALUE FOR HEMOPHILIACS	AVERAGE VALUE FOR 5 HEMOPHILIA-LIKE SWINE
Coagulation time of low speed plasma.....	90-125 sec.	111 sec.	165-540 sec.	255 sec.
Coagulation time of high speed plasma.....	105-145 sec.	171 sec.	330-900 sec.	429 sec.
Coagulation time of plasma after standing 6 hours.....	Small reduction from above value	126 sec.	Approaches normal	141 sec.
Whole blood coagulation time .....	Less than 8 min.	6.2 min.	More than 8 min.	14.0 min.
Quick's prothrombin time.....	11.5 sec.	9.1 sec.	12 sec.	9.2 sec.
Duke's bleeding time.....	Less than 4 min.	2.9 min.	Less than 4 min.	2.7 min.
Start of clot retraction.....	Less than 60 min.	68 min.	Less than 60 min.	58 min.

TABLE 4  
*Therapeutic value of blood globulin fraction*

ANIMAL NO.		AMOUNT INJECTED PER LB. LIVE WT.	FIBRIN PRECIPITATION TIME		REMARKS
			Before injection	1 hr. after injection	
17	To prevent hemorrhage after castration	mgm. 5	min. 48	min. 28	Hemorrhage not excessive
16	To establish potency of globulin	10	41	23.5	Preparation active
8	To arrest ear hemorrhage	5	50		Hemorrhage stopped in one hour
1	To prevent hemorrhage after castration	10	28	19	Hemorrhage not excessive
18	To establish potency of globulin	10	37.5	29	Preparation active
14	To arrest a mouth hemorrhage that had been bleeding 48 hours	10	35	22.5	Hemorrhage stopped in two hours

abnormality as we have observed it does not differ in any significant way from human hemophilia, except in swine the defect is not sex-linked.

In forming an opinion as to the nature of the abnormality a few additional observations may have some significance. When there is no visible injury to explain the hemorrhage the most common indication of the abnormality is blood

dripping from the mouth. In a few cases the site of the hemorrhage is the socket of a loosened tooth. It is not uncommon to observe bleeding from the defective animal's nose but it is difficult to determine the source. Hemorrhage and swelling in the joints also occur, especially when the animals are kept on a concrete floor. Other regions subject to bleeding are: 1, ears, snout, and tail; the result of minor injury. 2, navel; hernia cases. 3, vagina; following coitus or parturition. 4, intestinal wall; may result from injury by intestinal parasites. 5, rectum; producing bloody stools. According to Mills (7) the most common sites of spontaneous hemorrhage in hemophilia are from the mouth or nose and into the joints.

Quick (8) devised a method for the diagnosis of hemophilia in man and set up certain criteria for the blood of a true case of hemophilia. In order to apply Quick's criteria to our animals five bleeder and five normal swine were tested by his methods, and our data are shown in table 3. The parallelism is close though the swine seem to be more mildly affected than the hemophiliacs.

A considerable number of valuable experimental animals have died from loss of blood, therefore an attempt was made to find a therapeutic measure that would reduce the coagulation time of the blood and arrest the hemorrhage. The blood-globulin fraction, prepared from normal swine blood by the method of Pohle and Taylor (9), has been very useful. It is our practice to inject, intramuscularly or intravenously, 2 ml. of a saline solution containing 5 to 10 mgm. of dried globulin substance for each pound of live weight. The whole-blood coagulation time, or fibrin-precipitation time, were not reduced to the normal range but in practically every case they were markedly lowered and the flow of blood was arrested. Typical examples are summarized in table 4.

#### SUMMARY

1. A strain of swine was encountered in which the blood clotting mechanism is defective.
2. The fibrin precipitation time in diluted plasma is a reliable test for the abnormality.
3. The characteristics of the disease fit very closely the criteria set up by Quick for the diagnosis of hemophilia.
4. The injection of a globulin fraction prepared from normal blood reduces the coagulation time of the blood of abnormal animals.

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# THE ANOMALY OF A NORMAL DUKE'S AND A VERY PROLONGED SALINE BLEEDING TIME IN SWINE SUFFERING FROM AN INHERITED BLEEDING DISEASE<sup>1</sup>

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Hogan, Muhrer and Bogart (1) have described a bleeding disease in swine that is transmitted by both sexes as a Mendelian recessive (2). Examination of the blood of affected animals (1) shows normal concentrations of prothrombin, calcium, and fibrinogen; whole blood coagulation time is prolonged, and Duke's bleeding time is normal. Studies on the platelets indicate that they are abnormally stable, though present in normal number.

In a search for additional differences, we have found that affected animals have a prolonged Doettl and Ripke (3) bleeding time. In order more accurately to evaluate this anomalous finding, bleeder animals and suitable control animals have been tested at weekly intervals for a period of 3 months with both the Duke and the Doettl and Ripke methods. The results show a marked difference in the response of the affected animals to the two bleeding time tests.

**METHODS.** *Doettl and Ripke's bleeding time.* A modification by Copley and Lalich (4) was used. A lancet wound near or on the edge of the shaved ear to a depth equivalent to that used for Duke's test (1-2 cm. blot in 30 sec.) was considered acceptable. Care was taken to avoid small veins. The wound was immersed in 0.9 per cent NaCl solution brought to 37° in a 1 liter beaker. The bleeding time was the time that elapsed between lancet puncture and the cessation of any visible flow of blood from the wound. Duke's bleeding time: A lancet wound was made on the edge of the shaved ear and the blood was blotted at half-minute intervals with coarse filter paper until the flow had ceased.

The animals chosen for the 3 month experiment are described in table 1. All were purebred Poland Chinas belonging to one of 3 inbred lines of swine (lines 1, 2 and 3). Female 74 (line 1) was normal, but had produced bleeder offspring when bred to a bleeder boar. The other controls were from lines 2 and 3, inbred lines in which the bleeding disease has never been observed.

**RESULTS.** A summary of the weekly tests is given in figure 1. The curves on the left show that Doettl and Ripke's bleeding time is prolonged consistently in the affected animals. In normal animals bleeding stops promptly, usually in less than 100 seconds. The average bleeding time is 443+ seconds in the bleeders and 74 seconds in the normals for the 3 month period. There is therefore more than a 6-fold prolongation in the bleeders. The test seems to indicate those periods during which the affected animals are in greatest danger of hemor-

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TABLE 1

Description of animals used in the three month experiment

BLEEDERS	AGE	WEIGHT	NORMALS	AGE	WEIGHT
	mos.	lbs.		mos.	lbs.
F80.....	18	354	F74.....	18	342
F126.....	6	195	F68.....	6	203
			F139.....	6	229
M1.....	16	400	M81.....	18	570
			M30.....	7	276

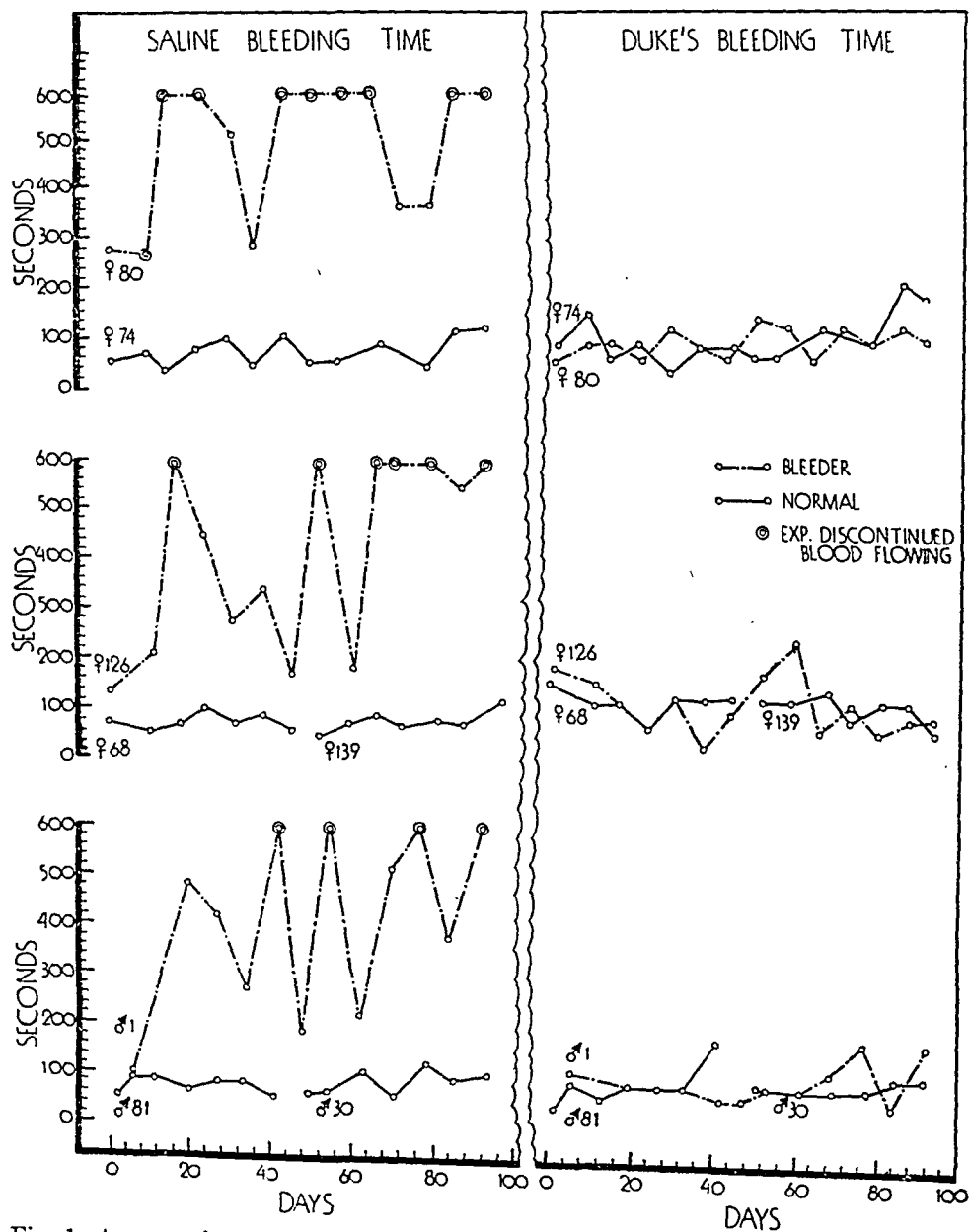


Fig. 1. A comparison of Duke's, and Doettl and Ripke's method, of determining bleeding time.

rhage. Thus, female 126 had a severe mouth hemorrhage on the 17th day. The saline bleeding time was over 10 minutes. On the 55th day, male 1 had a saline bleeding time of more than 10 minutes, and shortly thereafter almost bled to death from a superficial cut in the floor of his mouth. Both animals required special treatment (thrombin and pressure) to stop the flow of blood. Throughout the observations hemorrhages were observed repeatedly from the gums of the 3 bleeders at the points that came in contact with the rope used to tie them up. Mouth hemorrhages were never observed in the normal controls.

Readings were made on the bleeders for a maximum period of 10 minutes and in about 50 per cent of the tests blood was still flowing when the experiment was discontinued. We have not determined the upper limit of the bleeding time because of the difficulty in keeping the animals quiet.

The curves on the right (fig. 1) show that Duke's bleeding time is normal in the affected animals. The average Duke's time is 103 seconds in the bleeders and 102 seconds in the normals for the 3 month period. Six bleeders and 7 normal animals whose ages vary from 4 to 6 months, and in addition the animals listed in table 1 (except F68 and M81), have been tested by Duke's method, using Kleenex tissue instead of coarse filter paper. The average Duke's time was 173 seconds in the bleeders, and 195 seconds in the normal animals. The use of a highly absorbing paper apparently tends to prolong Duke's bleeding time, due perhaps to the more effective removal of surface blood. The lengthening occurs to the same extent in both the normal and the bleeder animals, however. The average saline time was 446+ seconds in the 6 young bleeders, and 77 seconds in the 7 young control animals.

DISCUSSION. The saline bleeding time is a comparatively new test, and only a few observations have been made by that method. According to Copley and Lalich (4) the average in normal men is 95 seconds. No values have been published for the saline time in the human hemorrhagic diathesis. In view of our findings with swine, the clinical use of the saline test may prove of value in the further classification of the bleeding diseases.

#### SUMMARY

The saline bleeding time in swine suffering from an inherited bleeding disease is about 6 times the normal value. In contrast, Duke's bleeding time is consistently normal.

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# THE EFFECT OF BODY SIZE UPON ENERGY EXCHANGE IN WORK

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Winslow and Gagge (1941) have found that a large man and a small man are about equally efficient in performing work on a bicycle ergometer but that the large man has an advantage over the small man in dissipating the excess heat produced in the work. The present study consists of a comparison of the energy exchange of a large man with that of a small man in grade walking and running on a motor driven treadmill.

The men used as subjects in the study were *EU* age 22, weight 99 kgm., stature 184.5 cm., and *MT* age 24, weight 61 kgm. and stature 170 cm. They were both excellent athletes with thin waist lines and no excessive fat deposits. The experiments were performed in an indoor laboratory in midsummer with the men clad in trunks, socks and tennis shoes. Both were accustomed to outdoor activity in the heat. Subject *EU* was particularly well acclimatized to heat by having operated a lawn mower on the university grounds for several hours each day and by having pitched 1 to 3 baseball games each week. The men were compared in 3 experiments in which the dry bulb temperature of the room ranged from 31.1 to 32.3°C. and the humidity from 68 to 74 per cent (table 1). In experiment 1 each man walked on the treadmill for 75 minutes at 5.6 km. per hour on an 8.6 per cent grade, an intensity of work which raised the metabolism to about 7 times the basal level. In experiment 2 the men walked on the same grade at 6.7 km. an hour but subject *EU* was forced by approaching exhaustion to stop after 63 minutes of walking. In experiment 3 the men ran slowly at 10.5 km. an hour on the level which raised the metabolism 43 per cent higher than did the work of experiment 1. Subject *EU* was exhausted after 30 minutes of work in experiment 3. In experiment 4 the work was the same as in experiment 2 but the dry bulb temperature of the room was raised to 35°C., the humidity reduced to 44 per cent and 4 electric fans turned on the men during work to evaporate the sweat as completely as possible (table 1).

The following determinations were made in each experiment: 1. Metabolism at 10 to 20 minute intervals during work by collection of expired air and analysis of samples on the Haldane apparatus. 2. Heart rate recorded continuously by means of a cardiometer. 3. Rectal and skin temperatures at frequent intervals by means of thermocouples (Aldrich, 1928). 4. Weight loss by weighing the men before and immediately after work.

**RESULTS.** In experiment 1 the large man (*EU*) was never able to attain a balance between heat production and heat loss and became exhausted after 75 minutes of work. His skin and rectal temperatures and his heart rate continued to rise throughout the experiment (fig. 1). In contrast the small man (*MT*) reached a steady state with less than 1°C. rise in rectal temperature and a heart

rate of 130. The metabolic rates of the two men during work were almost identical when related to weight but in relation to surface area *EU* was 21 per cent higher than *MT* (fig. 1). The heat accumulated during work may be estimated approximately from the rise in body temperature using 0.83 for the specific heat of the body. If heat accumulated and mechanical energy released by elevation of the body in walking up the grade are deducted from the total metabolism the approximate rate of heat dissipation may be estimated. From these estimations we find that *EU* dissipated heat during work at an average rate of 243 Cal./m<sup>2</sup>. per hour or 5 per cent faster than the rate of 232 by *MT*. However if heat dissipation is related to weight the rate of *EU* is 16 per cent lower than that of *MT*, the average values being respectively 5.5 and 6.4 Cal./kgm. per hour for the two men. Thus loss of heat was determined largely by the surface area and heat production by the body weight. The data in table 2 show that weight loss during work, due to sweating and loss through

TABLE 1

*Average room temperature during the work experiments*

	SUBJECT	DRY BULB	WET BULB
		°C.	°C.
Expt. 1 {	<i>EU</i>	31.7	27.4
	<i>MT</i>	32.2	27.4
Expt. 2 {	<i>EU</i>	31.7	27.8
	<i>MT</i>	32.2	27.3
Expt. 3 {	<i>EU</i>	31.1	26.7
	<i>MT</i>	31.6	27.0
Expt. 4 {	<i>EU</i>	34.8	24.9
	<i>MT</i>	35.2	25.2

TABLE 2

*The average rate of weight loss during work*

	SUBJECT	DURATION MIN.	WT. LOSS IN G. PER HR.	
			Per kgm.	Per m. <sup>2</sup>
Expt. 1 {	<i>EU</i>	75	25.4	1120
	<i>MT</i>	75	20.1	731
Expt. 2 {	<i>EU</i>	63	25.2	1120
	<i>MT</i>	71	26.2	920
Expt. 4 {	<i>EU</i>	74	20.1	884
	<i>MT</i>	74	20.2	725

respiration, was considerably greater in *EU* than in *MT* both in relation to surface and to weight, the difference being associated with the observed differences in skin and rectal temperatures. Under the humid conditions of the experiment each subject was wet with sweat within a few minutes after he started work and sweat began to drip off onto the treadmill. This led us to believe that both subjects had as great a rate of evaporation as air conditions permitted. Under the existing conditions the slightly greater rate of heat elimination by *EU* in relation to surface area was due to his higher skin temperature which produced greater radiation (fig. 1).

The results of experiment 2 substantially confirm those of experiment 1. Subject *EU* was forced to stop after 63 minutes of work with skin and rectal temperatures of 37.6 and 40.7°C. respectively and a heart rate of 180 while *MT* was able to attain heat balance with a rise of only 1.1°C. in rectal temperature (fig. 2). Because the rate of walking was greater in this experiment metabolism

was on the average about 22 per cent higher than in experiment 1 (fig. 2). The averages were 9.88 and 9.72 Cal./kgm. per hour for *EU* and *MT* respectively, and 440 and 346 Cal./m.<sup>2</sup> per hour. After deducting heat accumulated and the energy of work as described above the average rates of heat dissipation were 6.15 Cal./kgm. per hour for *EU* and 7.60 for *MT*, a difference of 23 per cent. As in experiment 1 the rate of heat elimination was closely related to surface area the mean values being 274 and 271 Cal./m.<sup>2</sup> per hour respectively for the two men. Weight loss in *EU* was at the same rate as in experiment 1 which was apparently very close to his maximal rate of sweating because on the basis of skin and rectal temperatures the stimulus was considerably greater in experiment 2 (table 2). *MT* sweated faster in the second experiment than in the first though still less than *EU* in relation to surface area. These rates of sweating are as great as the highest records of Robinson, Dill, Wilson and Nielsen

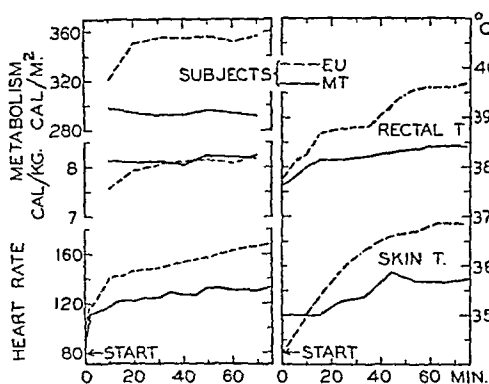


Fig. 1

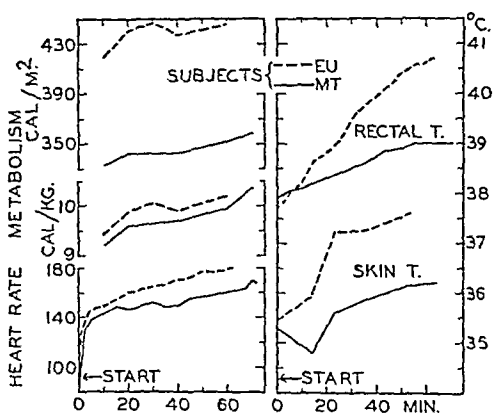


Fig. 2

Fig. 1 Records of body temperature, metabolism and heart rate of the subjects during experiment 1.

Fig. 2. Records of body temperature, metabolism and heart rate of the subjects during experiment 2. *EU* was exhausted and stopped after 63 minutes of the walk.

(1941) on a southern Negro performing similar work in a warm, humid environment.

The work and the environment in experiments 1 and 2 imposed conditions which overtaxed the capacity of *EU* to regulate his body temperature. With environmental conditions the same a further increase in the intensity of work was used in experiment 3 with the idea of reaching the maximal capacity of *MT* for temperature regulation. The results of the experiment are shown in figure 3 and reveal that even though his metabolic rate was 20 per cent higher than in experiment 2 and 43 per cent higher than in experiment 1 *MT* was able to approach a steady state in metabolism, rectal temperature and heart rate. He maintained the work for 40 minutes and apparently could have gone on much longer whereas *EU* was forced to stop after 30 minutes of work with a rectal temperature of 40.5°C. and a heart rate of 182. The metabolic rate of *EU* in relation to weight was only 6 per cent higher than that of *MT* at the start of



work but as his temperature rose during the 30 minutes of work the difference increased gradually to 23 per cent (fig. 3).

In experiment 4 the men worked at the same rate as in experiment 2 but the humidity was lower and fans were used to evaporate the sweat. The room temperature was higher than skin temperature after work started (fig. 4) therefore evaporation was the sole mechanism of heat dissipation. Each man attained heat balance and a steady state of heart rate during the experiment (fig. 4). The rectal temperature of *EU* reached a plateau  $1.7^{\circ}\text{C}$ . below his final temperature in experiment 2. He was slightly more efficient than *MT* and did not have an increasing metabolic rate during the progress of the experiment as in the preceding experiments this being associated with the fact that his body temperature did not increase so greatly as before. Skin temperatures of both men during work dropped markedly below dry bulb air temperature because the air movement and low humidity almost completely evaporated the sweat. It

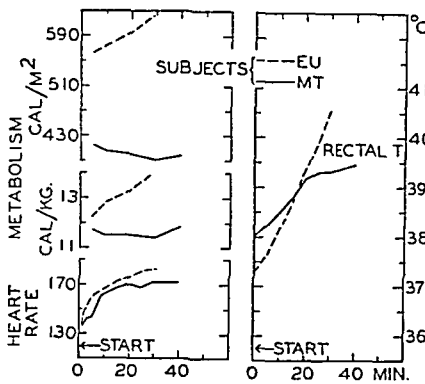


Fig. 3

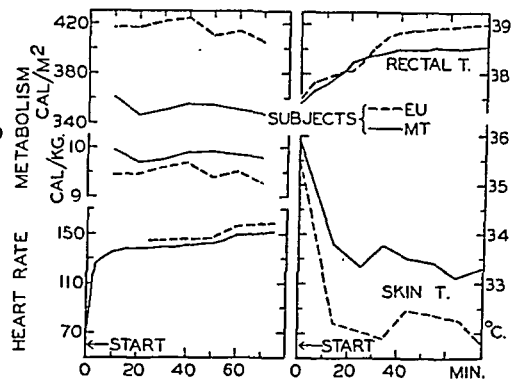


Fig. 4

Fig. 3 Records of rectal temperature, metabolism and heart rate of the subjects during experiment 3. *EU* was exhausted and stopped after 30 minutes of the run.

Fig. 4 Records of body temperature, metabolism and heart rate of the subjects during experiment 4.

may be observed in figure 4 that the skin temperature of *EU* dropped to an average of  $32.1^{\circ}\text{C}$ . after work began or  $1.5^{\circ}\text{C}$ . lower than the average of *MT* during work. This difference between the two men is accounted for by the fact that the water loss of *EU* was  $884\text{ g./m.}^2$  per hour and that of *MT* was only  $725$  (table 2). Since his skin temperature was lower *EU* dissipated  $310\text{ Cal./m.}^2$  per hour as compared with  $277$  by *MT*. The rate of sweating in relation to body weight was the same in the two men (table 2), a similarity which depends upon the fact that the total heat production was closely related to weight and the dissipation of this heat depended upon evaporation.

DISCUSSION. Winslow and Gagge (1941) found that the efficiencies of a large man and a small one in work performed on a bicycle ergometer were equal and that the increase in metabolic rate during work was not related to body weight but was proportional only to the work output on the ergometer. This

is true in this type of work because the subject does not have to move his body about in accomplishing the work. Since the large man's total surface area is greater and his total increase in heat production the same as the small man's for a given work output on the ergometer the large man can dissipate the extra heat more effectively.

The two subjects used in this study, a large man and a small one, were equally efficient in grade walking on the treadmill and since the work consisted entirely of moving and elevating the body the metabolic rates of both men during work at a given speed and grade were closely related to body weight. When the rate of walking was increased the further increase of metabolic rate was proportional to the body weight. The two subjects were greatly different in the ratio of weight to surface area the large man having 44 kgm. of weight per m.<sup>2</sup> of body surface as compared with 35 in the small man. Thus when both men walked at the same rate and heat production was proportional to weight the large man produced in relation to surface area about 20 per cent more heat than the small man. For this reason in these experiments where a warm humid environment restricted heat dissipation the large man was forced to store heat throughout periods of work in which the small man could easily attain heat balance. Similar differences would be found in such types of work as climbing, shoveling or even bicycling on the road where all or a large part of a man's work output consists of moving his body about. The army, as well as industry, might well improve efficiency on some jobs if these factors were considered in selecting personnel for work in hot environments. The important factors to be kept in mind are the ratio of body weight to surface area, the type and difficulty of the work, and the temperature and humidity of the environment.

#### SUMMARY

Two men ran or walked up a grade on a motor driven treadmill at rates which raised metabolism 7 to 11 times the basal level. One of the subjects weighed 99 kgm. and had 44 kgm. of weight per square meter of body surface and the other weighed 61 kgm. and had 35 kgm. per square meter. The efficiencies of the two men in performing the work were the same. In work which raised metabolism to 8 Cal./kgm. per hour, when heat dissipation was limited by a room temperature of 32°C. and a humidity of 70 per cent, the larger man accumulated heat throughout the work and was forced to stop with approaching heat exhaustion after 75 minutes. Under the same conditions the smaller man attained heat balance and a steady state of heart rate at a metabolic rate of 9.7 Cal./kgm. per hour. Both men in this type of work produced heat in proportion to body weight and since the ratio of weight to surface area was 20 per cent greater in the large man than the small man his heat production in walking at a given rate when related to surface was about 20 per cent greater. Therefore, since heat dissipation depended largely upon the skin surface, at rates of walking in which the small man could easily maintain heat balance under these environmental conditions the large man could not dissipate all of his body heat.

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# THE REACTION AND NEUTRALIZING ABILITY OF THE CONTENTS OF THE FIRST PART OF THE DUODENUM IN NORMAL DOGS UNDER FASTING CONDITIONS<sup>1</sup>

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The contents of the first part of the duodenum in normal dogs display an ability to neutralize, buffer and dilute gastric chyme which varies with the type of foodstuffs undergoing digestion, but which, nevertheless, is considerable (3, 4, 5). This study was undertaken in an attempt to determine whether a neutralizing efficiency obtained in the duodenal bulb under standardized conditions of fasting similar to that observed after different meals. Through the simultaneous determination of the various criteria of acidity in the pyloric antrum and duodenal bulb, we hoped to learn whether the physiologic relationships affecting the acidities in these contiguous areas were in any way modified from those observed during digestion under similar experimental conditions.

**METHOD.** Dogs were prepared with cannulated gastric and duodenal fistulas (7, 8) and then trained and maintained in the manner described previously (3). For a period of 36 hours preceding each experiment, and in a few instances for a period of only 24 hours, all food was withheld but water was permitted as desired. Each dog was placed on a table supported by a muslin hammock in a position which it had been trained to maintain. Following the insertion of the collecting and instillation tubes, during which process care was taken to cause as little trauma as possible, a rest period of 30 minutes was allowed for any unavoidable excitement or traumatic stimulation to subside. At the end of this period fractional samples were removed simultaneously from the pyloric antrum and the duodenal bulb at 10 minute intervals for  $\frac{1}{2}$  hour. As a rule all the material which could be obtained was collected at each aspiration.

The pH was determined on each gastric and duodenal sample without delay through the use of a Leeds-Northrup pH indicator which employs a glass electrode. The free and total acidity of each specimen was titrimetrically estimated after filtration, using Toepfer's reagent (dimethyl-amino-azobenzene) and phenolphthalein as the respective color indicators (2). On each duodenal sample, in addition, there was determined what was named the excess neutralizing ability. This consisted of the amount of N/10 hydrochloric acid which was required to cause a positive colorimetric reaction for free acid (2, 3).

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In all, 77 technically satisfactory experiments were performed on 7 dogs. These totaled 1437 different determinations which included 429 estimations of pH, 409 determinations of free acid, 399 determinations of total acidity and 200 estimations of excess neutralizing ability of the duodenal contents.

RESULTS. The quantity of material obtained from the stomachs and duodenums of the fasting dogs was variable. As a rule that gotten from the stomach was small, at times too small to make satisfactory pH readings. Duodenal secre-

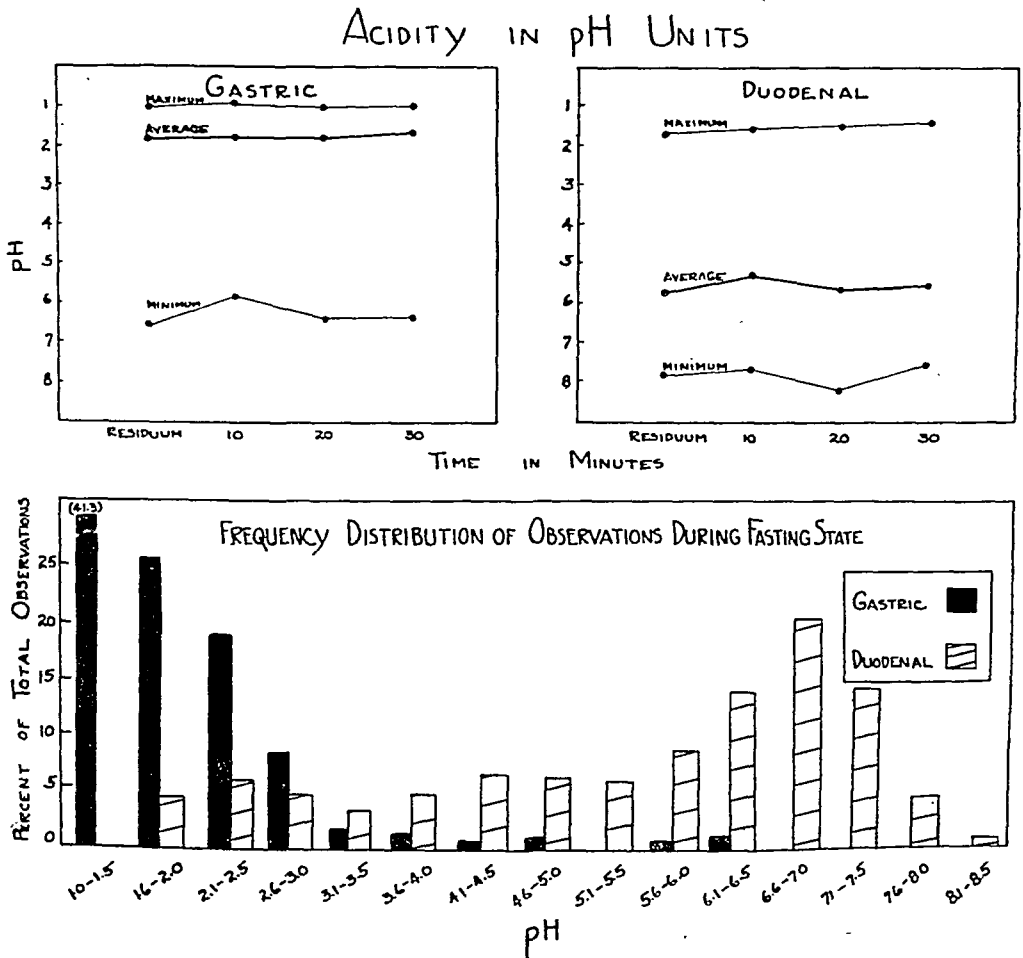


Fig. 1. Acidity in pH units of samples collected simultaneously from just above and just below the pylorus in fasting normal dogs.

tion, on the other hand, while far from profuse was usually greater in quantity than that obtained simultaneously from the stomach. Both the volume and reaction of the stomach contents were rather constant for each dog although individual variations were seen.

*Acidity in pH units* (fig. 1). *Stomach.* The average pH of the fasting gastric contents was fairly constant even though the individual specimens showed a wider range of variation (pH 0.99 to pH 6.5) than that observed following the use

of various gastric secretory stimuli. The average pH value during the fasting observation period (pH 1.90) was greater than that during the post-Ewald meal period (pH 1.63), but was decidedly less than those during the post-Liebig's extract-histamine (pH 2.01) and post-cream meal (pH 3.40) periods.

*Duodenum.* A very slightly changing average pH and a wide range of variation in the pH of the individual samples (pH 1.67 to pH 8.14) were also characteristic of the contents of the duodenal bulb. Fluctuation in the pH values of consecutive specimens was much more apparent than during the active digestion of various foodstuffs.

The ability of the contents of the first part of the duodenum to rapidly neutralize, buffer and dilute the secretions received from the stomach was as good, and in some respects superior, to that displayed during periods of digestion. A difference in pH was constantly maintained between the simultaneously obtained contents of the pyloric antrum and duodenal cap. Throughout the period of observation in the fasting state this averaged 3.66 pH units. Of all the fasting duodenal specimens 17.2 per cent had a pH of 3.5 or less, the value which we had adopted as the critical one for free acid (2). This percentage is just about the same as that after an Ewald meal (16.4 per cent) (3); it is less than that after a protein-histamine meal (26.5 per cent) (4); but it is greater than that after a fat meal (1.1 per cent) (5). On the other hand, the average fasting duodenal pH value was higher than the average pH value after any of the foodstuffs (fasting, pH 5.56; cream meal, pH 5.51; carbohydrate-water meal, pH 4.82; meat extract-histamine meal, pH 4.54).

*Free acid* (fig. 2). *Stomach.* The gastric free acid values in the fasting state closely simulated those following carbohydrate or meat extract-histamine stimulation both in average value and in range of distribution; they definitely exceeded the values obtained after the gastric secretory inhibitor, cream. The percentage of specimens colorimetrically negative for free acid (4.3 per cent) was not greatly in excess of the equivalent percentage after an Ewald meal (0.9 per cent) but was much less than after cream (53.2 per cent) or beef extract-histamine (18.6 per cent) meals.

As in the case of pH, the average gastric free acid values were fairly constant while wide fluctuations in individual values from determination to determination were not uncommon.

*Duodenum.* As evidence of the ability to rapidly neutralize the gastric secretion, the contents of the first part of the duodenum in the fasting state characteristically showed an absence of free acid. The percentage of samples colorimetrically positive for free acid (6.3 per cent) was less than the percentage (17.2 per cent) electrometrically positive (pH 3.5 or less) (fig. 1). This is attributable to the error introduced by our method of filtration and dilution preparatory to colorimetric titration which results in a number of false negative readings (2). The percentage of colorimetric free acid-positive duodenal samples, similar to the electrometric percentages, is nearly equal to that in the post-Ewald meal phase (2.6 per cent), and is less than that after beef extract and histamine (12.2 per cent). No free acid was observed colorimetrically after cream.

A nearly quantitative constancy was again displayed in the average values for duodenal free acid.

*Total acidity (fig. 3). Stomach.* The average gastric total acidity values were a little less than those after the more potent secretagogues but were greater than those following a fat meal. The range of distribution of the values was as wide as with any of the meals, although the averages were fairly constant.

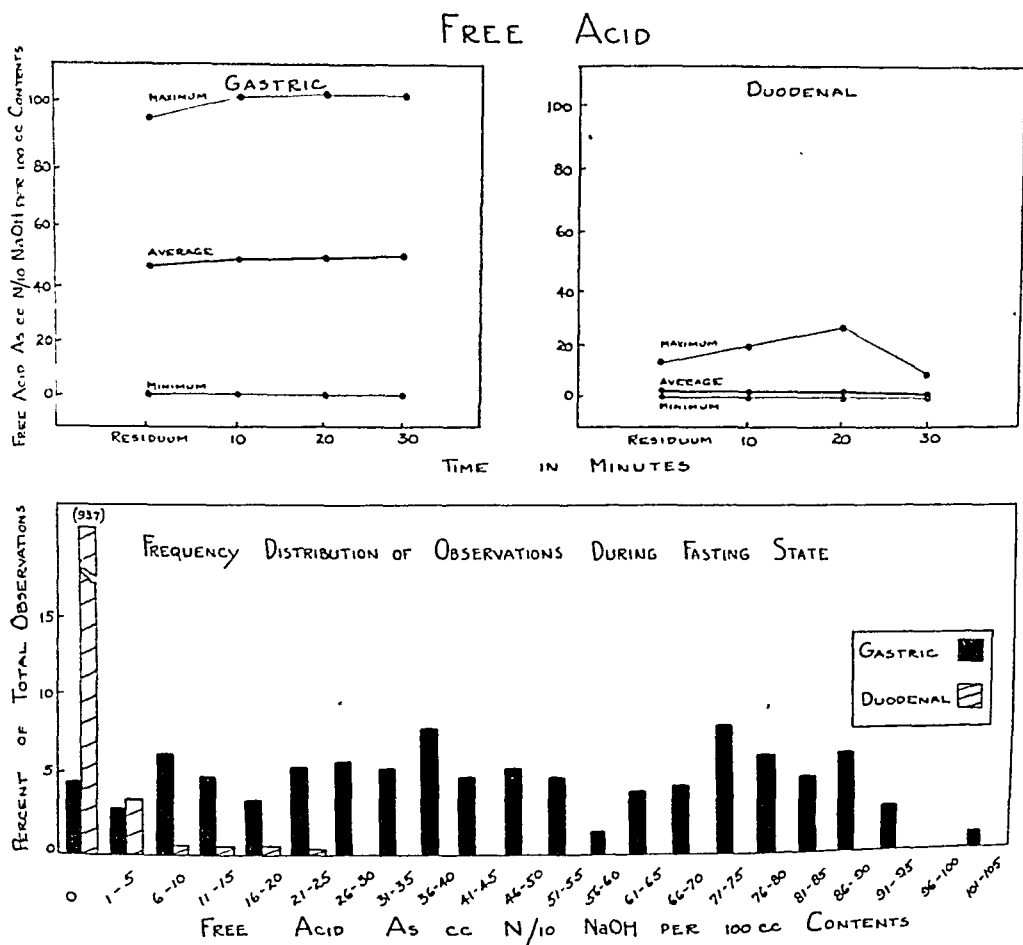


Fig. 2. Free acid as determined on samples collected simultaneously from just above and just below the pylorus in fasting normal dogs.

*Duodenum.* The efficient neutralizing mechanism in the duodenal bulb in the fasting state is evidenced again by the fact that the average value for total acidity in the duodenum during the entire period of observation (22 clinical units) was definitely less than comparable values for the entire post-meal phase following the various foodstuffs (carbohydrate-water, 30 clinical units; meat extract-histamine, 36 clinical units; cream, 47 clinical units). The range of the individual values was not as wide as during the digestive intervals with the exception of that after the Ewald meal. The average values, as in the stomach, were fairly constant.

*Excess neutralizing ability of the duodenal contents* (fig. 4). The excess neutralizing ability of the duodenal bulb contents has been defined as a measure of the reserve capacity which these contents possess to neutralize, buffer and dilute the material received from the stomach above that necessary to offset the free acid content (2, 3).

Both in the average value throughout the observation period and in the range of distribution of the values, the average excess neutralizing ability of the

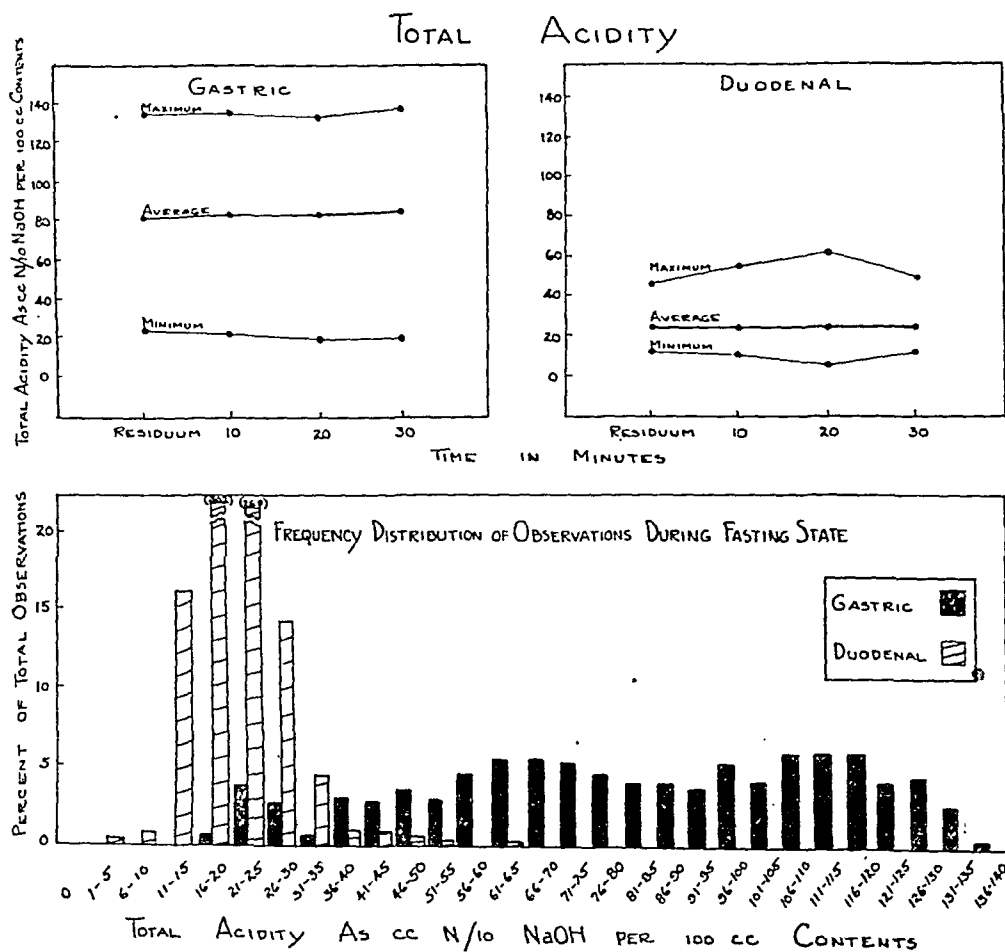


Fig. 3. Total acidity of samples collected simultaneously from just above and just below the pylorus in fasting normal dogs.

duodenal contents compared well with that seen following the use of different foodstuffs. In these respects it closely resembled the findings after the Ewald meal, a tendency which was noted with all the other types of determinations.

Of all the observations in the fasting state, 8.0 per cent failed to show (colorimetrically) any excess neutralizing ability, whereas 3.0 per cent of the post-Ewald meal and 16.6 per cent of the post-Liebig's extract-histamine samples were similarly negative; all of the post-cream meal specimens showed some excess neutralizing capacity.



DISCUSSION. Many normal and abnormal stimuli sufficient to influence gastric secretion may have existed in our dogs (1) despite our attempt to avoid as many of them as possible and to secure a true basal state. Consequently, the quantitative values for fasting gastric acidity which we obtained are not to be interpreted as necessarily normal values. Nothing, however, militates against their usefulness in affording a comparison with similar values simultaneously obtained in the first part of the duodenum or with corresponding values obtained in the digestive state under similar experimental conditions.

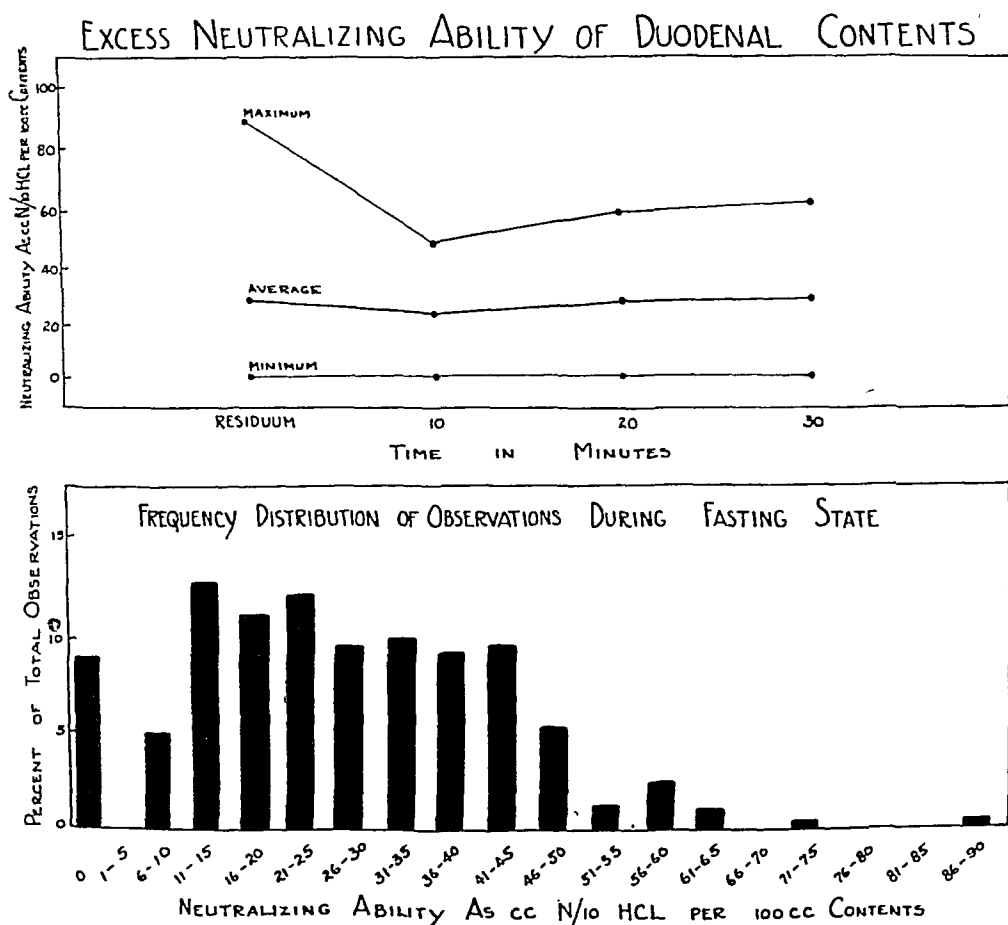


Fig. 4. Excess neutralizing ability of the duodenal contents in fasting normal dogs

Although variations occur, nevertheless, the volume and the acidity of the stomach contents in the fasting normal dog appear to be more or less characteristic for each animal just as various gastric secretory stimuli produce responses which are fairly characteristic for each.

A striking feature of the fasting state is a tendency for consecutive observations to fluctuate widely in value both in the stomach and duodenum. As a consequence, the pH values, in particular, vary through a range that is wider than that observed during periods of digestion. In spite of the fluctuation of the individual readings, the average values for all of the several determinations ex-

hibit an arresting uniformity. These characteristic tendencies for the values to show wide individual fluctuation but aggregate relative constancy are the most prominent features which distinguish fasting observations from those made during the active digestion of various foodstuffs.

The contents of the first part of the duodenum of normal fasting dogs display an ability to neutralize, buffer and dilute the gastric secretions which is equal to, and in some respects even surpasses, that seen during the digestive state. A difference in reaction is constantly maintained between the contiguous pyloric antrum and duodenal bulb which, on the average, is greater than that during digestion. That effective neutralization (ability to neutralize gastric free acid manifested by the ability to maintain a pH of 3.5 or above) is well maintained is shown by the over-all average duodenal pH of 5.56 which is higher an over-all average pH than that following various meals; still, approximately 1 out of every 6 (17.2 per cent) of the duodenal samples contained free acid (pH 3.5 or less).

It is of interest to note that the average hydrogen ion concentration in the stomach of fasting normal dogs is much greater than that in normal fasting people studied in an analogous manner (pH 1.90 in dogs; pH 3.51 in people) (6). The average duodenal hydrogen ion concentrations, in contrast, are about equal (pH 5.56 in dogs; pH 5.60 in people). It may well be that this superior neutralizing capacity in the duodenal bulb of dogs in part underlies the rarity of spontaneous duodenal ulcers in dogs on the one hand, and the frequency of this disease in humans on the other.

#### SUMMARY AND CONCLUSIONS

1. The contents of the first part of the duodenum of normal fasting dogs display an ability to neutralize, buffer and dilute the gastric secretions which is equal to, and in some respects even surpasses, that seen during the digestive state.

2. The volume and acidity of stomach contents in the fasting normal dog appear to be more or less characteristic for each animal.

3. A tendency for the various criteria of acidity in the stomach and duodenum to show wide individual fluctuation but aggregate constancy are the most prominent features which distinguish fasting observations from those made during the active digestion of various foodstuffs.

4. The over-all average pH in the stomach of fasting normal dogs (1.90) is much less than that in fasting normal people (3.51); the over-all average pH values in the duodenal bulb, nevertheless, are about equal (dogs, pH 5.56; people, pH 5.60).

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# HYPOTHALAMIC STIMULATION YIELDING ADRENALIN REVERSAL EFFECTS

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It has been shown that both pressor and depressor effects may be obtained on hypothalamic stimulation. While sympathetic and parasympathetic vascular responses have been attributed to different areas of the hypothalamus (Beattie, 1932), Kabat, Ranson and Magoun (1935) did not find a definite anatomical subdivision of vasopressor and depressor reactions. Hare and Geohegan (1939) noted that variations of frequency of stimulation altered the blood pressure response. Bronk, Pitts and Larrabee (1941) stressed the importance of afferent impulses and the pre-existing blood pressure level on the nature of the sympathetic discharge obtained on stimulation of the hypothalamus. While much attention has been directed toward the type and location of the neural response, there has been comparatively little consideration of the humoral aspects. In this investigation we wish to point out that adrenalin released by hypothalamic stimulation is an important factor in the determination of the character of the resultant blood pressure curve in the cat. The completely denervated iris was used as an indicator of circulating adrenalin. The pupil of such a preparation in the cat is very sensitive and dilates with intravenously injected adrenalin in concentrations of  $1 \times 10^{-8}$  per kilo of body weight (Bender and Weinstein, 1940).

**METHOD.** Cats and monkeys (*Macaca mulatta*) were used in these experiments. The iris was completely denervated (C.D.) by sectioning the optic and all ciliary nerves behind the globe. The arterial tension was recorded from a cannulated femoral artery. The animals were mounted in the Horsley-Clarke apparatus and the hypothalamus stimulated with a bipolar electrode after the technic described by Ranson (1934). Current was supplied through a thyratron condenser circuit. The frequency range used was between 12 to 250 per second. All stimulations were carried out under light nembutal anesthesia. In many instances the anesthesia was so light that when the stereotaxic apparatus was removed the animal appeared to be almost fully awake.

**OBSERVATIONS.** a. *Hypothalamic stimulation in the cat.* Two types of blood pressure response were observed—neural and humoral. The neural reactions were characterized by an abrupt rise in arterial tension which occurred after a latency of 1 to 2 seconds. At times the increase was as much as 100 mm. Hg. Paralleling this pressor response was a prompt dilatation of the normal pupil and retraction of the normal nictitating membrane (N.M.). These were parts of a sympathetic neural response. The humoral effect appeared 8 to 15 seconds

<sup>1</sup> Aided by grants of the Josiah Macy, Jr. Foundation and the Dazian Foundation for Medical Research.

after the application of the stimulus and was characterized by a slow dilatation of the c.d. iris to the maximum diameter. This was a consistent and spectacular phenomenon. The increase in the pupillary diameter varied from 1 to 6 mm. depending on the initial status; the maximal diameter was 13 or 14 mm. The mydriasis persisted for over 60 seconds after the cessation of the stimulus. Simultaneous with the onset of the mydriasis in the c.d. iris, the blood pressure began to fall as much as 40 mm. Hg. There was no apparent change in the pulse rate. The blood pressure continued to drop during the stimulation period, which was usually for 60 seconds. However, the normal pupil remained dilated, the nictitating membranes retracted, and the pupil in the c.d. iris maximally dilated. When the stimulus was removed, the normal pupil and the nictitating membrane quickly returned to prestimulatory status, while the pupil of the completely denervated iris remained at maximal dilatation, returning slowly to its initial diameter after 90 to 120 seconds. With cessation of stimulus, the fall in blood pressure was often accelerated and a deep dip below the base line resulted. The depressor effects were never accompanied by bradycardia, were not blocked by atropine and were not potentiated by eserine.

These effects were best produced with frequencies ranging from 40 to 250 per second. They could be obtained by stimulation of points throughout the entire hypothalamus in both anterior and posterior divisions. The reactive foci were situated in the region surrounding the third ventricle and were most consistently seen at coördinates 1 to 4 mm. lateral to the midline and from 6 to 8 mm. below the zero horizontal plane. The more lateral hypothalamic areas, though yielding good neural effects, were less productive of humoral responses. In other instances, the initial pressor action was not observed; instead there was an initial drop in blood pressure, retraction of the nictitating membrane, dilatation of normal pupil and after the usual latency of 8 to 15 seconds, a further drop in the arterial tension this time accompanied by dilatation of the pupil in the c.d. iris. In several animals, drops in blood pressure occurring 3 to 5 seconds after the start of the stimulus were obtained, but these were not accompanied by mydriasis in the denervated iris and were considered to be neural effects.

b. *Effects of adrenalectomy.* Unilateral adrenalectomy did not alter the results of hypothalamic stimulation but bilateral adrenalectomy or acute section of the thoracic spinal cord abolished the delayed mydriatic effects and almost completely obliterated the delayed drop in blood pressure. The disappearance of the delayed vasodepression was not a consistent phenomenon. In some instances the fall in blood pressure though not pronounced was found even after bilateral adrenalectomy. The pupil in the c.d. iris did not dilate even when very strong current was used. The immediate abrupt rise in blood pressure, the dilatation of the normal pupil and the retraction of the normal nictitating membrane were preserved and maintained during the period of stimulation. In some cats in which there was no initial pressor effect, hypothalamic stimulation after bilateral adrenalectomy had no influence on the blood pressure level, the curve remaining almost flat.

c. *Effects of adrenalin.* Some of these findings suggested that the stimulation of secretions from the adrenal glands were responsible for some of the delayed fall in arterial tension. Further proof was found when low concentrations of saline extract of the excised adrenal gland were injected intravenously into the adrenalectomized cat. These reproduced the humoral response obtained on hypothalamic stimulation, i.e., 1, a drop in blood pressure, and 2, a dilatation of denervated iris six to eight seconds after the intravenous injection. In order to quantitate these results, commercial adrenalin (Parke-Davis and crystallin form of Winthrop) was used. It was found that 0.01 gamma per second per kilogram of body weight injected intravenously for 3 to 5 seconds in a 1 cc. volume duplicated the humoral effects of electrical stimulation of the hypothalamus. Simultaneous stimulation of the hypothalamus and intravenous adrenalin injections in the above concentrations produced in the adrenalectomized cat the same pupillary responses and blood pressure curves as in the normal. Larger doses of adrenalin yielded only pressor effects. No delayed pressor effects coming on eight or more seconds after the onset of stimulus were observed. The delayed depressor response was neither blocked by atropine nor enhanced by eserine.

d. *Hypothalamic stimulations in the monkey.* In the monkey the synchronous delayed mydriatic and depressor effects were not observed. Frequent depressor as well as pressor reactions and pupillary dilatation in the normal eye were obtained, but the vascular responses were apparently neural. In contrast to the cat, conspicuous pupillary "humoral" effects were rarely seen. On one occasion, a slight mydriasis was noted in the denervated iris after an intramuscular injection of cocaine. In another monkey delayed mydriasis was obtained under urethane anesthesia. Adrenalin blood pressure reversal effects were not observed. Doses of 0.10 to 0.01 gamma of adrenalin injected intravenously produced a rise in blood pressure. Smaller doses had no effect on the arterial tension.

DISCUSSION. The above experiments confirm the fact that adrenalin is liberated on electric stimulation of the hypothalamus in the cat as previously demonstrated by Houssay and Molinelli (1925), Beattie, Brown and Long (1930). Magoun, Ranson and Hetherington (1937) used the sympathetically denervated nictitating membrane as the indicator for adrenalin in similar experiments. From our own experience we find the nictitating membrane, following excision of the superior cervical ganglion, is not nearly as reliable an indicator for adrenalin as the c.d. iris possibly because the membrane in such a preparation is only partially denervated. Adrenalin not only affects the pupil but also influences the blood pressure curve. In small quantities intravenously injected adrenalin was shown by Elliot and Durham (1906) to produce a drop in blood pressure. It is not surprising, therefore, to note delayed vaso-depressor effects on hypothalamic stimulation. These depressions, along with the delayed mydriasis, are abolished by adrenalectomy.

The amount of adrenalin discharged on hypothalamic stimulations under nembutal anesthesia is probably on the order of 0.01 gamma per kilo per second.

This was estimated by determining the quantity of intravenously injected commercial adrenalin necessary to reproduce the mydriasis and fall in blood pressure obtained on hypothalamic stimulation. Such small quantities of adrenalin reproduce the syndrome of delayed mydriasis in the c.d. iris and a drop in the blood pressure. It is probable, however, if extremely strong electrical stimuli were applied, larger quantities of adrenalin might be liberated and the delayed depressor effect would be replaced by a pressor reaction. Houssay and Molinelli (1925) on stimulating the hypothalamus of the dog estimated 8 to 15 gamma of liberated adrenalin, which gave a marked and sustained rise in blood pressure. Stewart and Rogoff (1924) found that stimulation of one splanchnic nerve in the cat under ether anesthesia resulted in the secretion of adrenalin at the rate of 0.013 gamma per second per kilo of body weight. The amounts of adrenalin liberated during rage or struggle, however, are difficult to quantitate. In three of our experiments the stimulation of an adrenergic point in the hypothalamus with an ensuing delayed mydriatic and depressor response was accompanied by the so-called pseudoaffective rage components. When the anesthesia was very light, stimulation produced violent struggle, rhythmic panting, meowing, hissing, spitting, dilatation of the pupil in the c.d. iris, and surprisingly a drop of 10 mm. Hg in the blood pressure. We were unable to estimate the amount of adrenalin liberated during these stimulations but judging from the duration of the mydriasis in the c.d. iris it must have been more than 0.01 gamma per second per kilo.

It may be that in different emotional states, varying quantities of adrenalin are liberated, the larger yielding pressor and the smaller depressor effects in cats. It is also likely that more than one hormone is secreted during physiologic activation of the hypothalamus. Clark and Wang (1939) found evidence for the release of a pressor hormone from the posterior lobe of the pituitary, while Magoun, Ranson and Hetherington (1937) were able to detect a discharge of sympathin on electric stimulation of the hypothalamus in the cat.

#### SUMMARY

1. Hypothalamic stimulation in the cat produces simultaneously delayed depressor and mydriatic effects. These responses are humoral and are due to liberation of small amounts of adrenalin.

2. The dilatation of the pupil in the denervated iris and some of the delayed vaso depressions thus obtained can be abolished by adrenalectomy and reproduced by intravenous injection of adrenalin.

3. Some of the delayed falls in blood pressure are due to the liberated adrenalin—an adrenalin reversal effect.

4. Adrenalin reversal effects in the monkey could not be demonstrated either by hypothalamic stimulation or intravenous injections of adrenalin.

We are indebted to Mr. M. Grabiner for his technical assistance in this problem.

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# THE INFLUENCE OF POSTURE ON BLOOD FLOW IN THE DOG<sup>1</sup>

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When subjects are placed in the upright position there is a marked retardation of blood flow in the lower extremities (1). This slowing appears to be due primarily to stagnation or pooling in the veins. The present study was designed to give further and more direct information concerning the blood flow changes with posture as measured by the thermostromuhr method in different parts of the body.

Sixty successful experiments were carried out on 20 dogs lightly anesthetized with chloralose (40 to 80 mgm. per K. B. W.) or Na barbital (180 to 250 mgm. per K. B. W.). They were placed in a trough which was rotated above a transverse horizontal axis, the position of which was adjusted in each case to coincide with the axis of the cannula used for recording the blood pressure from the carotid or the femoral artery. The distance from the point of arterial cannulation to the fourth interspace (heart level in the upright position) was measured and the value thus obtained applied as a correction for the hydrostatic pressure effect to all blood pressure readings made with the animal in the upright position. The animal was prevented from slipping when in the upright (75°) position by tying the mouth securely around a bit fixed to the animal board. Respiration was measured by means of a pneumograph. Blood flow was measured by means of direct current thermostromuhurs (2) in the femoral artery and vein, the renal artery and vein and the carotid artery and jugular vein. The units were connected to a high sensitivity Leeds and Northrup galvanometer through a double-throw switch which made it possible to make alternate (and practically simultaneous) observations of the flow in pairs of vessels or to study the flow in each vessel in successive experiments without disturbing the position of the thermostromuhurs. The units chosen for application to the vessels to be measured were of such size as to fit the vessels snugly and cause slight constriction. The usual procedure was to take a short control in the horizontal position after which the animal was tilted to an angle of 75°, feet down, and kept in this position for periods varying from 3 to 20 minutes before being returned to the horizontal. The tilting usually took about two seconds. Measurements of the flow were usually continued for at least 15 minutes after the animal was returned to the horizontal position. It was found impossible to obtain accurate readings of the changes in flow as the animal was tilted or immediately thereafter. This may have been due to rapid changes in temperature of the blood as a result of its sudden redistribution (3). Some difficulty was also experienced in obtaining

<sup>1</sup> Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.



venous blood flows when the respiration was very deep or fluctuated considerably. When this occurred, average readings were usually recorded. Considerable variation in the response to tilting was encountered due to the practical impossibility of achieving the same level of anesthesia in different animals as well as to variations in their compensatory respiratory and vasomotor mecha-

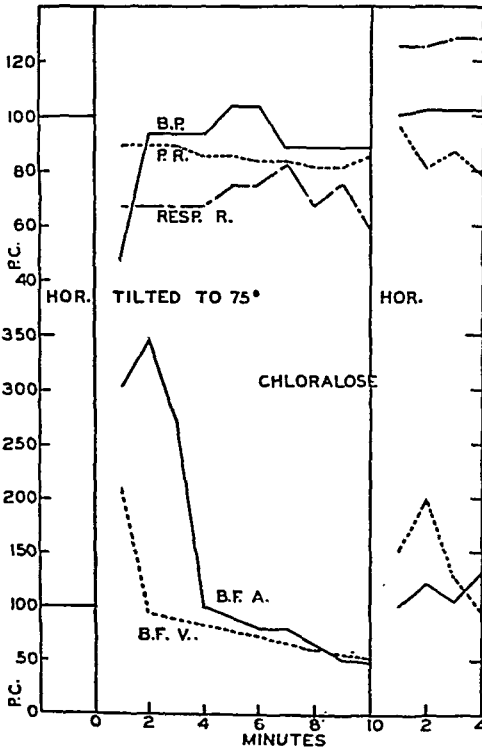


Fig. 1

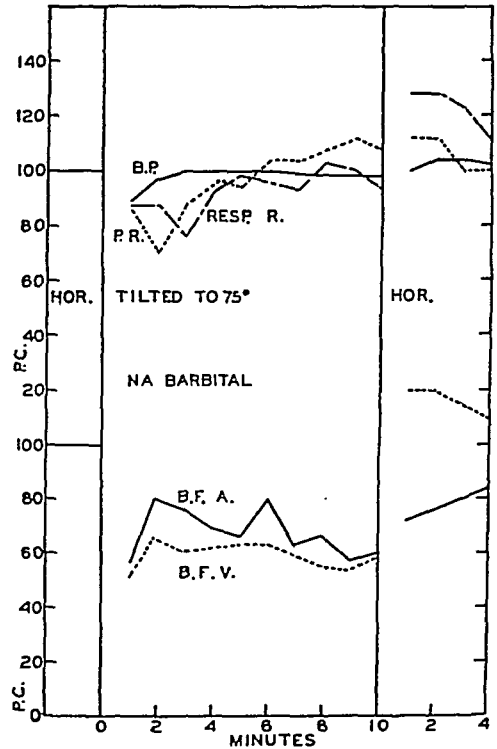


Fig. 2

Fig. 1. Percentage changes in blood flow in the femoral artery, *B.F.A.* and vein, *B.F.V.* Blood pressure, *B.P.*, recorded from opposite femoral artery and corrected for hydrostatic pressure effect. *P.R.* = pulse rate; *Resp. R.* = respiratory rate. Control values: *B.P.* = 145 mm. Hg; *B.F.A.* = 56 cc. per min.; *B.F.V.* = 42 cc. per min.; *P.R.* = 126 per min.; *Resp. R.* = 12 per min.

Fig. 2. Percentage changes in blood flow in the carotid artery, *B.F.A.*, and jugular vein, *B.F.V.* Blood pressure, *B.P.*, recorded from opposite carotid artery and corrected for hydrostatic pressure effect. *P.R.* = pulse rate; *Resp. R.* = respiratory rate. Control values: *B.P.* = 160 mm. Hg; *B.F.A.* = 67 cc. per min.; *B.F.V.* = 30 cc. per min.; *P.R.* = 267 per min.; *Resp. R.* = 14 per min.

nisms. This was particularly true of the changes for the first few minutes after tilting and after return to the horizontal position.

The ranges of blood flows in the various vessels were similar to those found by other investigators using the same or different methods (4). No differences were observed between the two anesthetics. With the animal in the horizontal position, control blood flows in cubic centimeters per minute in different experiments varied as follows: femoral artery—42 to 166; femoral vein—40 to 132;

carotid artery—23 to 189; jugular vein—40 to 148; renal artery—53 to 360; renal vein—44 to 248.

Typical changes in the rates of flow in the femoral artery and vein when the animal was tilted are shown in figure 1. The fall in intrafemoral pressure, evident immediately or within a minute after tilting, was usually associated with an increase in arterial and venous flow. Within several minutes, however, the rate of flow in the vein dropped sharply so that at the end of 10 minutes the rate of flow was only 12 to 64 per cent of the control rate before tilting. In several experiments the flow in the vein was almost stopped at the end of this period. The flow in the femoral artery showed similar changes except that the decrease usually came several minutes later and was never quite as marked as in the vein, the values at the end of 10 minutes being 50 to 84 per cent of the control rates. These results, indicating a slowing of the blood flow in the extremities, have been corroborated by determination of the oxygen saturation of the arterial and venous blood and the finding of a definite and often marked increase in the arterio-venous oxygen difference (5).

The rates of flow in the carotid artery and jugular vein decreased immediately after tilting and remained low except for a few instances where there was a secondary rise lasting from 3 to 5 minutes (fig. 2). At the end of 10 minutes the rates of flow had decreased to 50 to 90 per cent of the control rates in both vessels. These changes were usually related to those in carotid blood pressure and were accompanied by a marked decrease in oxygen saturation of the blood in the jugular vein (5). Loman and Myerson (6) reported similar changes in jugular blood flow in man.

The changes in flow in the renal artery and vein were less consistent. In the six experiments in which the rate of flow was measured in the renal artery, three showed results similar to those given in figure 3. In two experiments there was no significant change in blood flow over a ten-minute period while in the sixth experiment the rate of flow diminished with the assumption of the upright position but rose to the control value after 3 minutes. Likewise, results similar to those depicted in figure 3 (decrease to 30 to 40 per cent) were obtained in 3 of 4 experiments in which the rate of flow was measured in the renal vein. No changes were observed in the fourth experiment. In one experiment, renal vein flow diminished from 124 to 5 cc. per minute at the end of 10 minutes in the upright position. Smith (7) studying the diodrast and inulin clearances in man, found similar changes with posture. The failure of the filtration fraction (inulin clearance divided by effective plasma flow) to vary inversely with the renal blood flow was interpreted as indicating a constriction of afferent arterioles.

In the majority of experiments the rates of flow in all the vessels approximated the pre-tilting values within 6 to 10 minutes after the animals were returned to the horizontal position. In some instances the flow immediately after the animal was returned was considerably higher than the control value, dropping gradually to the pre-tilting level after 3 to 4 minutes.

In a previous publication (8) we reported that denervation of the carotid sinuses or cutting the vagi, thus eliminating the two aortic nerves, uniformly

diminished the animal's ability to compensate for gravity. Elimination of both pressor receptor mechanisms often resulted in complete absence of compensation and a consequent drop in blood pressure to shock levels from which there was slight recovery. The blood flow changes in the carotid artery in such an experiment are shown in figure 4. Similar changes were observed with respect to the femoral artery and vein and the jugular vein. In the absence of compensatory vasoconstriction the blood pressure and blood flow failed to show

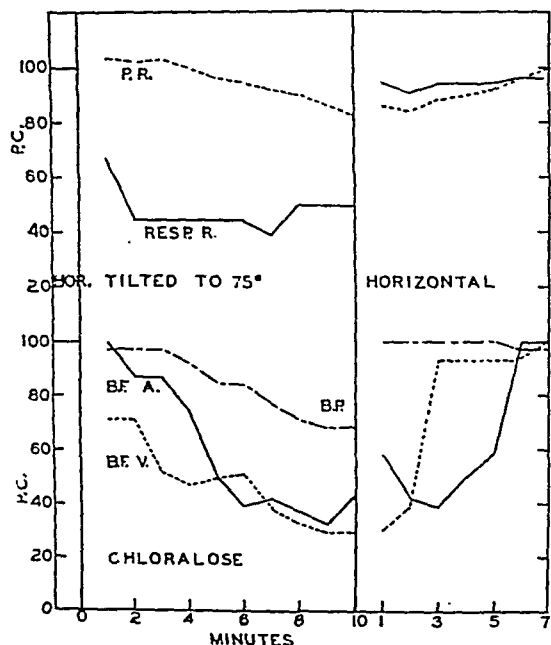


Fig. 3

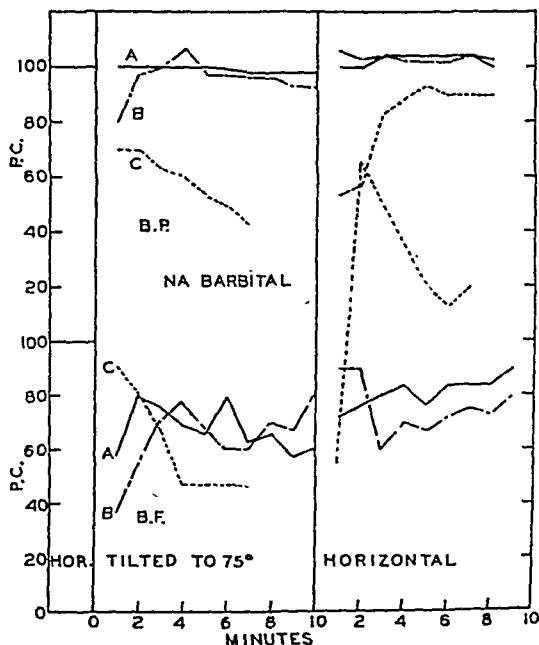


Fig. 4

Fig. 3. Percentage changes in blood flow in the renal artery, *B.F.A.*, and vein, *B.F.V.*. Blood pressure, *B.P.*, recorded from carotid artery and corrected for hydrostatic pressure effect. *P.R.* = pulse rate; *Resp. R.* = respiratory rate. Control values: *B.P.* = 150 mm. Hg; *B.F.A.* = 360 cc. per min.; *B.F.V.* = 248 cc. per min.; *P.R.* = 185 per min.; *Resp. R.* = 16 per min.

Fig. 4. Percentage changes in blood pressure, *B.P.*, and blood flow, *B.F.* in the carotid artery. Blood pressure corrected for hydrostatic pressure effect. *A*: intact animal; *B*: after carotid sinus denervation; *C*: after additional cervical section of vagi. Animal returned to horizontal at 7 minutes to prevent complete circulatory collapse. Control values: *A*:—*B.P.* = 165 mm. Hg; *B.F.* = 63 cc. per min.; *B*:—*B.P.* = 175 mm. Hg, *B.F.* = 67 cc. per min.; *C*:—*B.P.* = 205 mm. Hg, *B.F.* = 105 cc. per min.

their usual secondary rise, there being a gradual drop to dangerously low levels which necessitated the return of the animal to the horizontal position to prevent complete respiratory and vasomotor failure and death.

The results obtained in these experiments supplement and extend those previously reported on man in which measurements of circulation times in the upright position indicated a marked retardation of blood flow to and from the lower extremities (1, 9). They provide direct evidence that as a result of the com-

pensatory orthostatic vasoconstriction there is a slowing of the volume flow to the subcardial regions with consequent decrease in renal (7), gastric (10), and intestinal activity (11). This decrease in flow on the arterial side is accompanied by a similar decrease in venous return due to pooling and stagnation in the capillaries and veins which may be particularly marked if the upright position is maintained for more than relatively short intervals of time in the absence of adequate vasomotor and muscular tone (12). Under these conditions the carotid pressure and blood flow may be diminished to such an extent as to fail to meet the demands of the brain for oxygen and a relative anoxemia and consequent circulatory failure may ensue. The more marked changes in the rates of flow in the femoral and renal veins emphasize the possibility that circulatory failure, when it does occur, may be due primarily to weakness on the venous rather than on the arteriolar side of the system.

#### SUMMARY

Tilting of anesthetized dogs to the upright (75°) position, feet down, resulted in a consistent and marked decrease in the rate of blood flow in the femoral vein and artery and a less pronounced fall in the carotid artery and jugular vein. Changes in renal flow were less consistently observed, but in the majority of experiments were in the same direction.

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I am also indebted to Messrs. W. D. Davis, Jr. and W. J. Trautman, Jr. who assisted in performing some of the experiments.

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# EFFECT OF FASTING AND FEEDING ON THE RESPONSE OF SURVIVING TISSUES TO QUINIDINE

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The multiplicity of factors which modify the behavior of surviving isolated tissues and of enzyme systems necessitates a careful statement of each experimental situation. In this paper the necessity for stating the antecedent nutritional state of the animal, at least in the case of liver slices of the rat, will be demonstrated. The paper concerns the response of the surviving isolated slices of rat kidney and liver, in the presence of adequate antecedent food intake and after enforced fast, to the protoplasmic poison quinidine. This drug contains basic nitrogen in two functional senses and is isomeric with its levo stereomer quinine.

The metabolic actions of quinine on surviving isolated tissues were studied by Druckrey (1935) who reported the initial augmentation by the drug of the utilization of oxygen by "normal" liver slices. He ascribed the initial augmentation to tissue damage. Pennetti (1926), using the Lipschitz (1921) method, which employs dinitrobenzol, found that quinidine inhibits the utilization of oxygen by the surviving liver, kidney and nervous tissue of the dog. The complex nature of the reaction of quinine on the metabolism of various races of yeast has been stated by Rona *et al.* (1923, 1927).

Quinidine and quinine possess pharmacodynamic and therapeutic properties which differ chiefly in degree. Our results indicate that the similarity of action obtains also in the case of the respiration of certain surviving tissues.

**METHOD.** The Warburg technic (1926) using Dickens and Simer (1931) vessels was employed to measure the oxygen utilization by surviving tissue slices. After the attainment of initial control respiratory level, the drug to be studied, dissolved in normal saline, was introduced from the side-cup into the compartment containing the tissue slices.

Unlike Druckrey's experiments which employed  $\text{H}_2\text{CO}_3\text{-NaHCO}_3$  buffered Ringer's solution or inactivated horse serum, these experiments were carried out in a modified Krebs' (1933) buffered saline (pH 7.3) in which the  $\text{Mg}^{++}$  and  $\text{Ca}^{++}$  concentrations were halved. This medium contained also 0.2 per cent glucose as substrate. The outer well of the vessels contained KOH to absorb  $\text{CO}_2$ . The experiments were performed in an atmosphere of oxygen at a temperature of  $37^\circ \text{C} \pm 0.02$ . The values for  $\text{Q}_{\text{O}_2}$  were calculated against dry weight of tissue. The various concentrations of the drug used will need to be described in the results for each separate experimental situation.

**RESULTS.** *Action of quinidine on:* 1. *Slices of rat kidney cortex—fasted and non-fasted.* The results are shown in figure 1 plotted as concentration action

curves. The slices were brought to equilibrium and their oxygen utilization measured for ten minutes. The drug was then introduced. Beginning twenty minutes later the oxygen utilization was measured over the succeeding twenty minute period. Oxygen utilization during this period, *i.e.*, between the 30th and 50th minute after equilibrium (100 to 120 minutes after the death of the animal), was arbitrarily chosen for comparison with the utilization during the control period. In no experiment was there more variation than five minutes in either direction from the specified time for initiating the experimental periods. The ordinates of the curve were calculated by dividing the oxygen utilization during the arbitrarily chosen period of drug action by the oxygen utilization determined at the outset and multiplying by 100 per cent. Since these percentages are necessarily modified by a depression of oxygen utilization arising

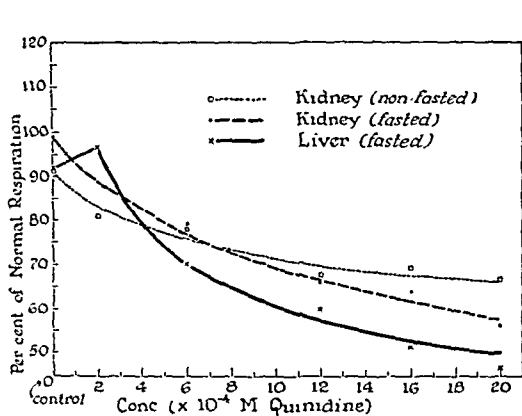


Fig. 1

Fig. 1. Effect of quinidine on the respiration of rat tissue slices, expressed as concentration-action curves. The time selected for the experimental values was from the 20th to the 40th minute after the addition of the drug. The curves for kidney tissue represent an average for five experiments, for liver three experiments.

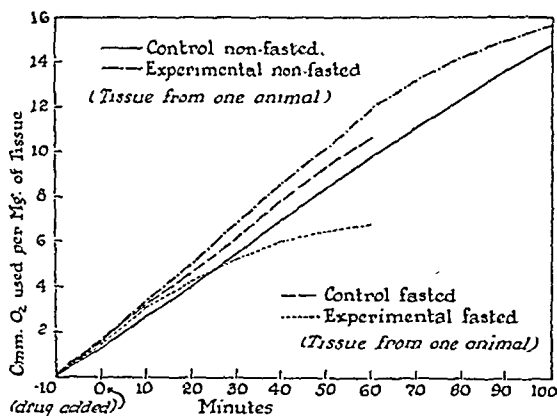


Fig. 2

Fig. 2. Summated oxygen utilization by slices of rat liver (fasted and non-fasted) in the presence of  $20 \times 10^{-4}$  M. quinidine.

from unavoidable tissue deterioration, they are compared in the graph to an ordinate for zero concentration of quinidine (control) in which the percentage response was calculated by comparing the oxygen utilization of untreated slices during the experimental period with that of the control period.

The idealized curve for kidney slices obtained from rats fasted 24 hours represents averages of five experiments. It indicates a depression of about 40 per cent at a concentration of  $20 \times 10^{-4}$  M quinidine. Such a curve for non-fasted kidney slices representing averages of 5 experiments indicates a depression of about 20 per cent for the same concentration. We consider this to be a significant difference.

2. *Liver slices—fasted rats.* The animals were fasted 24 hours before death. The concentration action curves for these slices arrived at by the method used on kidney slices is similar to the curve for kidney slices; a depression of about

40 per cent was produced by the highest concentration of quinidine (fig. 1). This is about the highest concentration which the limited solubility of quinidine permits in the presence of phosphate buffer ( $M/75$ , pH 7.3). The curve also indicates, even in the case of fasted animals, a stimulation of respiration with the lowest concentration of quinidine used. It was drawn from the averages of three experiments.

3. *Liver slices—non-fasted rats.* The action of quinidine on liver slices from non-fasted rats is not amenable to representation by concentration-action curves because it is a dual one consisting of augmented and inhibited phases. The curves of figure 2 represent two typical experiments (one fasted and one non-fasted) drawn by plotting the sum of the increments of oxygen utilization per milligram of dry tissue per ten minutes. The control period is repre-

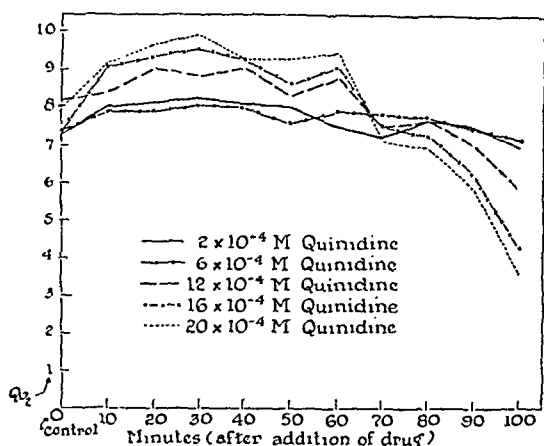


Fig. 3

Fig. 3. Composite time-action curves showing the effect of quinidine on the oxygen utilization of non-fasted rat liver. The curve for each concentration of drug is an average of results from three experiments.

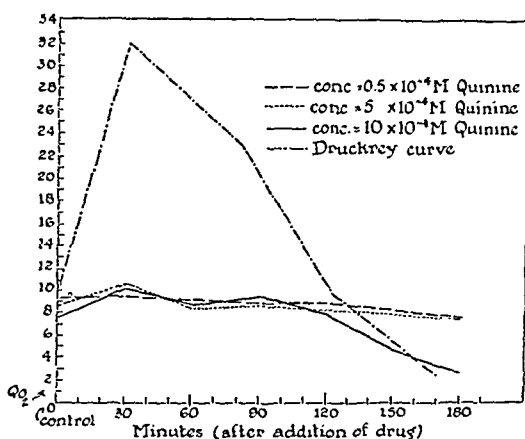


Fig. 4

Fig. 4. The effect of quinine on the respiration of non-fasted rat liver slices; comparison with Druckrey's results.

sented by the abscissa from  $-10$  minutes to zero minutes, at which latter time the drug was added to the system. To avoid the confusion of too many lines the results of only the control and highest concentration are graphed for each experiment. Comparison of these curves shows clearly that quinidine initially augments and finally depresses the respiratory metabolism of non-fasted rat liver slices, an effect differing from that with fasting tissue where only depression was observed with this concentration of the drug. The results of these experiments are in excellent accord with those of sixteen other experimental situations. In figure 3 the average time-action curves for three experiments emphasize the dependence of metabolic augmentation upon drug concentration.

*Action of quinine on:* 1. *Rat liver slices—non-fasted.* The responses of non-fasted liver slices in modified Krebs' solution (pH 7.3) to quinine are plotted in figure 4 as time-action curves in which the ordinates represent  $Q_{O_2}$  values and

the abscissas represent time intervals. Results obtained in concentrations from  $0.5 \times 10^{-4}$  M. to  $10 \times 10^{-4}$  M. are compared with those reported by Druckrey (1935) who used carbonic acid-bicarbonate buffer and concentrations of quinine like ours. His procedure included a preliminary treatment of the tissues with  $5 \times 10^{-5}$  M. quinine, which concentration was subsequently augmented during the experiment to reach  $5 \times 10^{-4}$  M. His observation of a 200 per cent initial augmentation followed by final inhibition contrasts sharply with our augmentation of only 20 per cent followed also by depression. The results of our experiments with quinine are similar to our results with like concentrations of quinidine. The limited solubility of quinine prevents the use of concentrations higher than those we report. Our results with quinine on fasted rat liver are like those obtained with quinidine. They are not shown in the graph.

*Results with intact animals.* We were unable to demonstrate any significant difference between the LD 50 of quinidine sulfate on intact fasted and non-fasted rats. This drug intraperitoneally injected is lethal to 50 per cent of both groups of rats in doses of 105 mgm./kgm. The action on the intact animal is characterized by convulsions terminating in respiratory embarrassment and arrest. Although the convulsive seizures can be controlled by barbiturates, death eventually supervenes. These data were gained through the use of 24 rats.

**DISCUSSION.** The observations herein reported indicate the importance of inclusion of a statement of the antecedent nutritional state of animals which are used to furnish tissue slices for the study of respiratory metabolism. The presence of glucose substrate in the experimental vessel in no wise minimizes this necessity. The importance of the effects of adequate prior feeding becomes evident when the slices are poisoned by a drug. The difference may be overlooked if the oxygen utilization by unpoisoned slices from fasted animals is compared with that of unpoisoned slices from non-fasted animals. In such situations, providing glucose substrate be present, there seems to be little significant difference between the initial oxygen utilization by fasted and non-fasted tissue slices.

In this study a lapse of two hours after death of the animal has been construed as liminal for satisfactory observation of the gaseous metabolism of surviving tissue slices. If forced by the necessity of comparison with the observations of other investigators, we have, of course, prolonged the time of observation.

It seems that the quinidine induced preliminary augmentation of oxygen utilization by non-fasted liver slices and the contrasting inhibition in the case of fasted slices are related either to the presence of ample glycogen in non-fasted slices or to modification of the detoxicating properties of the fasted slices by the act of fasting or by both. Detoxication might lead to the formation of a derivative of quinidine which agreed in action with the parent drug neither qualitatively nor quantitatively. It is not chemically unthinkable that either quinidine or some detoxication product of it might "compete" with the apoenzyme for iron-porphyrin prosthetic group of cytochrome oxidase.



It is not the purpose of this paper to attempt complete reconciliation of our results in which specificity of action was sought with those which Druckrey reported as essentially non specific. The fact that, in this study, augmentation of respiration of non-fasted liver slices was obtained with quinidine, whereas depression resulted in the case of fasted liver slices, points to the achievement of specific action. The differences between our medium and Druckrey's, particularly with respect to the presence of glucose in ours, may have caused the difference. The divergency cannot be related to a misunderstanding of what Druckrey meant by "normal" animals because liver slices from neither fasted nor non-fasted animals yielded in our experiments such a strikingly large augmentation of respiration as he reported quinine to have produced. Limitations of physical equipment prevented our study of the obviously important glycolytic phenomenon.

The difference between our results and those of Druckrey may also have arisen from the fact that in these experiments  $\text{PO}_4$  buffer was used, whereas he relied primarily upon bicarbonate buffer. The concentration of the phosphate in the medium used in these studies ( $\text{M}/60$  before the addition of the drug and  $\text{M}/75$  after the addition) would appear to be low enough so as not to produce damage to the tissues. Dickens and Simer (1931) have shown that the respiration of a variety of tissues is the same in bicarbonate saline medium as in a  $\text{M}/40$  phosphate saline medium. Van Heyningen (1935) showed that at a phosphate concentration of  $\text{M}/100$  the reaction of tissues to cyanide is the same as in bicarbonate saline medium. Further, the qualitatively different response of fasted tissue as compared to non-fasted tissue would also preclude the idea of tissue damage as the cause of the observed results. Although any attempt to interpret the results in terms of the concept of phosphate bound energy (Kalckar, 1941; Lipmann, 1941) would seem to be premature, the implication of that concept in interpreting prevention of tissue damage and in detoxication is rationally possible.

#### CONCLUSION

1. The oxygen utilization by the surviving tissue of rat liver in the presence of glucose substrate and the ions of Krebs' solution is initially augmented by quinidine when the animals have not been fasted; it is depressed when the animals have been fasted.

2. The oxygen utilization by the surviving tissue of rat kidney is depressed by quinidine. The action is qualitatively the same for the tissue of fasted and non-fasted animals.

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# THE INFLUENCE OF BENZEDRINE ON WORK-DECREMENT AND PATELLAR REFLEX

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Voluntary muscular fatigue in man has long been studied by the taking of ergograph records of the maximal voluntary contractions of the middle finger of the hand. Using such a method, Mosso (1890) delineated many of the phenomena of voluntary muscular fatigue, and later (1893) observed an increase in voluntary work capacity following the administration of small doses of caffeine. While there have been several later studies of the effects of caffeine upon voluntary muscular work, the effects have been so inconstant and slight that it is not entirely clear today what conditions do determine an increase in work capacity after caffeine administration. However, some of the reported observations do indicate that the central nervous system stimulant effects of caffeine are dominant in the increase in the capacity for voluntary muscular work. The object of the present investigation was to determine the influence of another central nervous stimulant, benzedrine, upon the capacity for voluntary muscular work, and to relate its effects on work capacity to another central nervous system stimulation phenomenon that could be coincidentally observed. In this connection, observations of patellar reflex activity were made during the rest intervals between observations of the work capacity of the middle finger.

**EXPERIMENTAL STUDIES.** A Mosso-type ergograph, in which a weight was lifted by the middle finger and a kymograph record made of the movement of the weight, was used. The index and ring fingers were inserted into two rigidly anchored metal tubes, and the forearm was strapped in place on the base of the apparatus. The weight was kept constant during any given series of experiments, and the contraction effort was made in time with a metronome operating at 36 beats per minute until no further effective movement of the weight occurred. The subject was instructed to make a maximal contraction effort, involving flexion of all the joints, with each beat of the metronome, as Reid (1928) found that, outside of some modification in the character of the fatigue curve profile, little difference was found between the tracings made with the first or first and second interphalangeal joints in restraint and those in which free movement of all phalanges was permitted.

For study of patellar-reflex activity, a chair was designed in which the seat sloped to the rear to produce a maximum tension on the patellar tendon, and the back made a wide angle with the seat to afford a semi-reclining position to facilitate complete relaxation. An electromagnetic hammer positionally adjustable to the individual was used, and its frequency and intensity of stroke were con-

trolled by a condenser-discharge timer. The amplitude of the movements of the foot was recorded with a kymograph or a pen-writing polygraph.

Observations were made on six normally healthy adult males ranging from 22 to 38 years in age, and each series of observations on an individual was begun in the morning, one to two hours following a light breakfast, and was continued without further food intake until completed. Initially, the subject relaxed in the reflex chair until a steady state appeared to have been reached, then the initial recording of patellar-reflex activity was made. Following this, the subject walked a few paces to the ergograph and made a work capacity record, then returned to sit relaxed in the reflex chair until the time of the next observation.

In the initial studies, the ergograph was loaded with 2.7 kgm. and periods of 30 minutes between observations were used with all six subjects. One or two series of normal observations without any drug administration were made. Then a series of observations was made in which, immediately after the initial observations of patellar-reflex activity and work capacity, there was taken orally 10, 20 or 40 mgm. of benzedrine sulfate with a small amount of water. The initial observations thus furnished a basis for comparison of work capacity and patellar-reflex activity in the series on any one day, so that the work decrement or changes in reflex activity could be relatively valued with repeated observations.

Figure 1 shows graphs of this type of comparative study, parts A and B showing the decrement in work capacity of G. A. occurring in the repeated tests made during control periods of two hours on different days, during which the patellar-reflex activity varied but little. Parts C and D of figure 1 show the graphs of work capacity and patellar-reflex activity when a 20 mgm. dosage of benzedrine sulfate was taken. While the initial decrement in work capacity shown in C was much like that in A or B, the work capacity was increased at the 60-minute observation as compared with that at 30 minutes; and at the 90, 120 and 150-minute observations the work capacity was even greater than the initial work capacity. At these same times, the patellar-reflex activity was very notably increased, as shown in part D.

The observations made with the six subjects are summarized in table 1 with valuations of the work capacity and patellar-reflex activity made relative to the initial observations. The work values were compared by determining the areas of the kymograph tracings, since the kymograph speed, contraction frequency and load were constant throughout any experimental series. In the tabulation the initial work values were taken as 100, and succeeding work values expressed in per cents of this value. The initial reflex activity was given a rating of one plus and succeeding activities rated in comparison by using one or more plus values. As shown by these data, following a dosage of 10 mgm. of benzedrine sulfate there was an increase in work capacity, evident about 30 minutes later, though sometimes requiring 60 to 90 minutes, and even longer, before the effect was clearly evident. The usual effect was not to increase the work capacity above the initial level, but was simply an action against the decrement occurring in successive observations. The patellar-reflex activity was quite regularly observed to be increased at times of increase in work capacity or at times when the

work capacity was greater by comparison with the normal decrement of successive observations in the control series. Usually the higher dosages gave more definite effects with regard to increases in work capacity and patellar-reflex activity, but this was not always evident, particularly as shown by R. I., in whom 20 mgm. appeared to have less effect than 10 mgm. on work capacity.

In the course of doing the preceding experiments, it became apparent that, although all the individuals were leading regular and temperate lives and the details of observation were held as constant as possible, there was on occasion an unpredictable 40-70 per cent variance in the initial work capacity of these individuals. Correspondingly, the work decrement found on any one day would vary considerably as compared with that of another day, and it soon became apparent that some modification of technic was desirable to make clearer the effect of benzedrine sulfate upon work capacity or its decrement as studied.

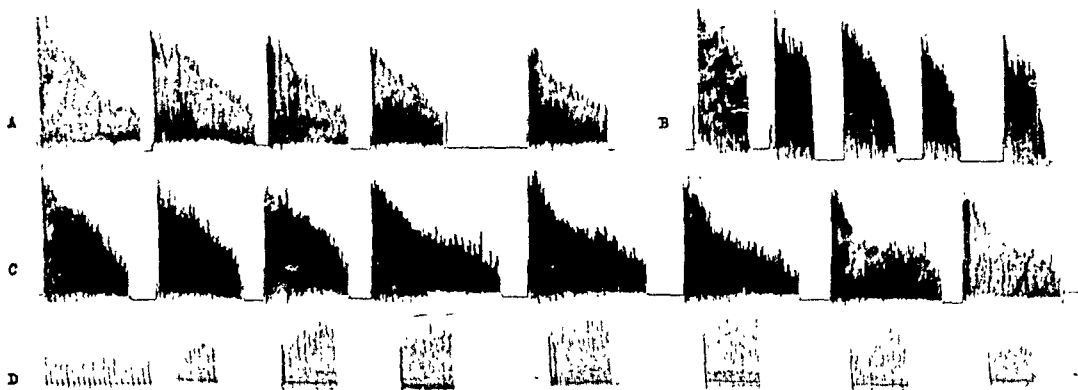


Fig. 1. A and B. Total finger work capacity on different days at successive trials every 15 minutes. C. Total finger work, and D, patellar reflex activity on another day at successive trials every 15 minutes following an initial taking of 15 mgm. benzedrine sulfate orally.

To obviate these difficulties, so far as they interfered with the clear demonstration of the effect of benzedrine sulfate upon work capacity, a different routine of observation of its effects was developed. This consisted of determining for each individual the effective load which, when observations were repeated every 15 minutes, would give a normal work decrement series in which the work capacity became negligible within a two-hour period. Then, at two hours, when the work capacity was at this level, administration of the drug and continuation of the work capacity trials (without interruption) demonstrated clearly the effect on work capacity, and permitted some estimation of the duration of that effect. Loadings of from 2.7 to 5.0 kgm. were required for this type of study with the same persons previously studied. Figure 2 shows the graphs of the work done with such an experimental procedure by G. F., before and after 10, 20 and 40 mgm. of benzedrine sulfate as used in the different experiments. It is to be noted that marked effects were present for three hours after administration of but 10

TABLE 1  
*Finger work capacity and patellar-reflex activity*

	BENZEDRINE SULFATE	TIME OF OBSERVATIONS IN MINUTES								
		Initial	30	60	90	120	150	180	210	240
G. A.	<i>mgm.</i>									
	0	100	86	92	76	78	—	—	—	—
	0	100 +	105 +	99 +	76 +	85 +	— —	— —	— —	— —
	20	100 +	87 ++	92 +++	122 ++++	128 +++++	103 +++++	94 ++++	85 ++++	— —
G. F.	40	100 +	132 ++	145 +++	107 ++++	105 ++++	109 ++++	98 ++	96 ++	— —
	10	100 +	79 +	46 +	34 +	32 ++	50 ++++	54 ++	— —	— —
	20	100 +	70 +	60 +	49 ++	58 ++++	29 ++	30 ++	— —	— —
	40	100 +	92 +	58 ++	71 ++	87 ++++	62 ++++	58 ++	50 ++	53 ++
P. S.	0	100	86 <sup>*</sup>	80	64	70	68	48	—	—
	0	100	108	102	100	85	88	75	—	—
	0	100	88	62	51	38	40	42	—	—
E. R.	10	100 +	122 ++	105 +++	14 +++++	9 +++++	44 ++++	34 ++	— —	— —
	0	100	110	102	75	60	55	64	—	—
	10	100 +	130 +	133 +++	92 ++++	89 ++++	76 ++	66 ++	— —	— —
R. I.	20	100 +	129 +	138 ++	137 ++	111 ++++	121 ++	80 ++	— —	— —
	0	100	61	13	5	3	5	4	—	—
	10	100 +	95 +	85 ++	49 ++	38 ++	47 ++	45 ++	— —	— —
C. B.	20	100 +	57 +	30 ++	9 ++++	5 ++	2 ++	1 +	— —	— —
	0	100	74	55	41	36	43	36	—	—
	10	100 +	130 ++	121 +++	98 ++	100 ++++	90 ++	94 +	— —	— —
	20	100 +	100 +	58 +	66 +	137 ++	138 +	127 +	— —	— —

Benzedrine sulfate taken orally after initial observation.

Work capacity in per cent of initial value—reflex activity in + of initial value.

mgm., and the 20 mgm. experiment was continued to five hours with no great further decrease in work capacity at this time as compared with the capacity shown at three hours. Similar experiments were also carried out with four of the other five subjects worked with previously, and with each individual similar striking recoveries of work capacity were demonstrated following dosages of 10 mgm. or more of benzedrine sulfate. These effects were evident in all cases within 60 minutes after administration orally. Increases in patellar-reflex activity were also evident at times of increased work capacity, and there was a fair time correspondence in the degrees of these two effects.

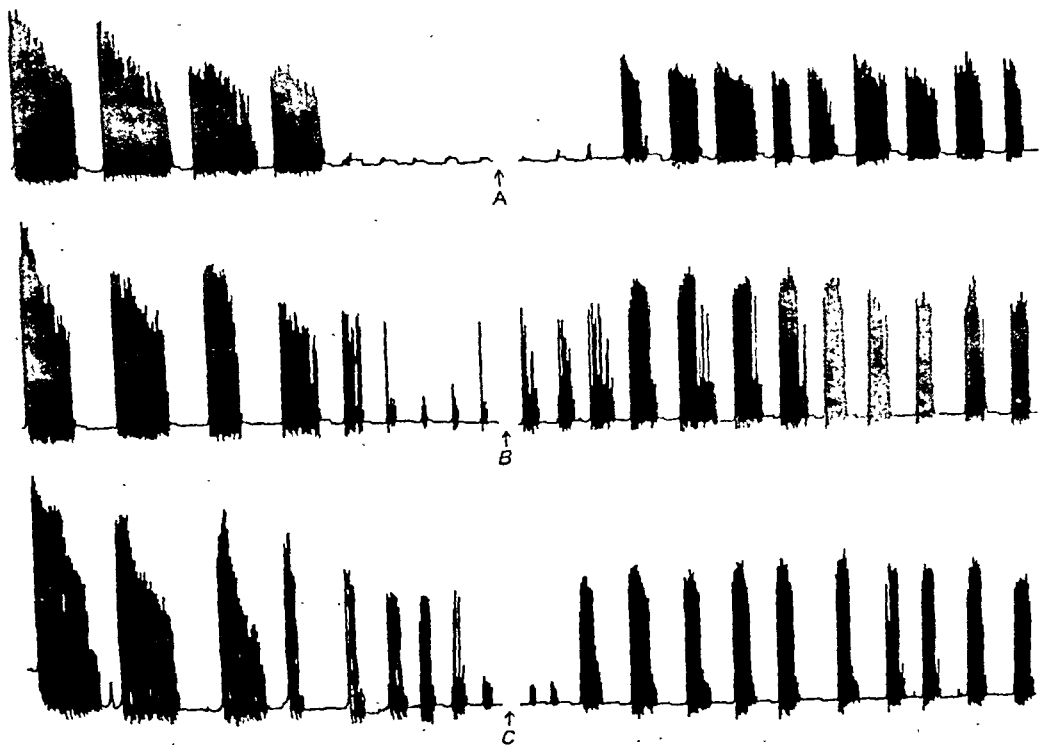


Fig. 2. Total finger work capacity on different days with benzedrine sulfate after initial two hours. In first series, at A, 10 mgm. administered orally after 8th test trial and the 9th test trial made on schedule 15 minutes later with successive trials continued for 3 hours after. In second series, at B, 20 mgm. were administered and in third series, at C, 40 mgm.

At the beginning of the present work it had been decided to use caffeine as a comparison drug for the fatigue studies. Preliminary experiments on the authors with dosages of 100, 200 or 400 mgm. of caffeine taken orally failed to show any definite results, the effects, if any, being within the normal daily variance. With the technic of first carrying out observations to a negligible work capacity level, it was considered possible to compare directly the effects of caffeine and benzedrine on fatigue. These attempts were carried out by orally administering, in different experiments, 100, 200 or 400 mgm. of caffeine (200, 400 or 800 mgm. of caffeine citrate) after the initial two-hour work decrement period. Then, after a

second two-hour or longer observation period of caffeine effects, a dose of 10 mgm. of benzedrine sulfate was orally administered and its effects on work capacity noted during a subsequent two-hour period. The results obtained were, however, rather equivocal and inconstant due to the influence of variables inherent in the training process.

The first series of experiments of the "exhaustion" type were performed using caffeine alone. In all tests included in this series the right hand was used and the weights were the same as those used for each subject in the previously described benzedrine experiments. The work capacity of G. A. was undiminished at the end of the 2-hour initial period in all tests of this series, and the various dosages of caffeine were incapable of increasing work output above normal. In the first experiment of this series with G. F. a low work-capacity level was approached at the end of 2 hours, and subsequent administration of 200 mgm. of caffeine citrate produced slight increase in subsequent work performance. In the following experiments, involving 400 and 800 mgm. of caffeine citrate, the initial 2-hour period did not result in work-capacities low enough to permit satisfactory basis for judgment concerning the influence of caffeine on the work curves following its administration at the conclusion of the initial 2-hour control period.

Failure to produce sufficient decrease in work capacity in the initial control period in the foregoing first series of experiments prompted the authors to change the hand undergoing fatigue, and to increase the loads used. In a preliminary experiment with G. F. where the working hand was changed from the right to the left, but the load left unaltered, no considerable decrement in work capacity occurred during the initial control period, although this was the first time that he had used that hand in an ergograph experiment. Under these conditions, the administration of 200 mgm. of caffeine citrate resulted in no change in the work curves during the continuation of the observational period.

Repetition of the experiment with an increased load produced complete exhaustion in less than 2 hours, which was not relieved by 200 mgm. of caffeine citrate in the two hours after its administration, but which subsequently responded to 10 mgm. of benzedrine sulfate with a marked increase in work capacity within 30 minutes of its administration. In the following experiment with the increased load, there also was complete exhaustion during the initial test period, and the administration of 400 mgm. of caffeine citrate restored the work capacity for  $4\frac{1}{2}$  hours before exhaustion again set in, and then 10 mgm. of benzedrine sulfate caused a marked restoration of work capacity for the additional  $3\frac{1}{2}$  hour period during which observations were continued. In another experiment, after the initial control exhaustion period, 800 mgm. caffeine citrate produced an increase in work-capacity for  $7\frac{1}{2}$  hours, but the side-effects of irritability and unsteadiness were rather extreme. Attempts to confirm these observations with regard to caffeine effects on work capacity were unsuccessful due to the fact that it was impossible to find a suitable load to produce consistently the initial degree of fatigue required. The final weight tried with the left hand, and which failed to produce the initial fatigue, was three times as great as the one which had been used with the right hand in the initial experiments cited in this paper.



In similar experiments with G. A., after changing to the left hand instead of the right and increasing the load, neither the 200 nor the 400 mgm. doses of caffeine citrate showed demonstrable effect, although the subsequent 10 mgm. dose of benzedrine sulfate showed quite a definite work-capacity effect in the continuation of the experiments. In the experiment involving the 800 mgm. dose of caffeine citrate, the work capacity did not decrease suitably during the initial control period, and in consequence no definite caffeine or subsequent benzedrine effects were observed. Increasing the load in a following experiment, under the same dosage regime, also failed to produce a definite result.

The amplitude of the patellar reflex was materially unchanged in all of the studies on both G. F. and G. A. following caffeine administration, though considerable jitteriness and unsteadiness were observed by G. F. following the higher doses of caffeine citrate.

Because of training and rapid adaptational factors, that may permeate any set of experiments of this type, very definite conclusions cannot be drawn as to the effects of caffeine upon work decrement. However, it is evident that, when therapeutically useful doses of both compounds are given in a single experiment, benzedrine sulfate is more effective than caffeine citrate in producing a return toward normal of voluntary muscular activity after "exhaustion".

The effects of training were well illustrated during the first part of the studies here reported in that, during the consecutive experimental trials on different days, there was fairly constant improvement with time, and this might exceed the apparent effects of administration of benzedrine sulfate or caffeine citrate on any one day. In the later experiments of the exhaustion type, this phenomenon was also noted when the subject failed to become exhausted with a given weight which had been adequate to bring his work capacity to a low value in the same time during the preceding experiments of a series.

The transfer of training from one hand to the other was also noted in these experiments, which may be considered to support the idea that the work capacity is limited by a central nervous system mechanism. In the case of G. F., at the beginning of the present ergograph studies which ran over a period of one and a half years, he was adequately exhausted within the initial two-hour period with a weight of 2.7 kgm. when using his right hand. As the experiments progressed, the loading had to be increased to 4.2 kgm. After adaptation to this load, the left hand was employed with the same weight, with resultant rapid adaptation, and at the conclusion of these experiments of this paper 8.5 kgm. was an insufficient load to produce decreases in work capacity levels during the initial control period.

**DISCUSSION.** The mechanism of action of drugs in increasing the capacity to do voluntary muscular work has chiefly been considered in the past with respect to the effects of caffeine. Mosso (1893) was the first to observe an increase in ergographic work following the administration of caffeine, but this phenomenon was much more extensively studied by Hoch and Kraepelin (1896) and by Osetzkowsky and Kraepelin (1901). These latter authors reported that caffeine acted to increase the extent of each contraction, but not the number of contrac-

tions, before fatigue. From the studies of Kraepelin and his co-workers it was considered to be established that the number of contractions was primarily related to the state of the nervous system, and their extent related to the state of the muscle. The conclusion was drawn that caffeine acts directly upon the muscle in producing its effect of increasing muscle work. Of the several studies on ergographic work effects of caffeine in man since carried out, the work of Rivers and Webber (1907) is of greatest interest in connection with proposed mechanisms of action. They found that in the two subjects extensively studied the effect on the number and extent of contractions was quite different, in that the one (W.) the effect was predominantly upon the extent of contractions, and in the other (R.) the effect was predominantly upon number, though both effects could be seen in either individual under particular conditions. This demonstrated that caffeine exhibited a double action in its effect on capacity for muscular work, and it was pointed out that it was well-known that caffeine acts on the isolated neuro-muscular mechanism and on the capacity for mental work. It thus appears that there is no definite conclusion yet to be drawn as to the exact site of action of caffeine in producing its effect on voluntary work capacity in man, though it often does exhibit a double action, and this doubleness of action may be related to dual mechanisms of action.

More recently the mechanism of voluntary muscular fatigue in man has been further investigated by Reid (1928) who was able to establish that any failure of the peripheral neuro-muscular mechanism is not an important factor in voluntary fatigue. Only with very rapid contraction rates under well-loaded conditions (120-160 per min., with 2 kgm. or more) may there be a peripheral effect overlapping the central cause of voluntary fatigue, while with a slow series (12 to about 80 per min.) there was little evidence for reduction of muscle contractile power. It is of interest, in connection with the present studies on benzedrine, that the blood supply to the muscle under conditions of extreme limitation can determine the voluntary muscular fatigue from serial contractions and that the earlier central failure is attributable to the influence of afferent inhibitory impulses from the working muscle.

In the present studies with benzedrine, the effects upon work production were not clearly either an increase in extent of contraction or an increase in duration of contractions, but rather an effect on both, though on occasion one or the other effect was dominant. In general, the effects upon duration of contraction were the more notable under the earlier experimental conditions, where benzedrine was taken immediately after the initial observations. When the benzedrine was taken after complete fatigue had set in, as in the later series of experiments, the effect on extent of contraction was the notable effect, the duration of such effects being quite constant over a considerable period of time. Benzedrine, like caffeine, thus appears to have a double aspect in its action upon voluntary muscular fatigue, but it is not clear that this is to be related to a duality of mechanism of action.

The fairly close correspondence in the present experiments of the increases in patellar-reflex activity with increases in muscular work capacity would indicate

that these are similar actions of benzedrine. While benzedrine exerts other central nervous system stimulant effects, those that are best known are usually of appreciably longer duration from the same dosages. The anti-sleep and mental stimulant activities resulting from 10, and particularly from 20 or 40 mgm. of benzedrine sulfate persisted through the succeeding night with most of the subjects of the present studies, though definite voluntary work or patellar-reflex effects usually passed through a maximum in from two to five hours after administration. While the higher doses (20 and 40 mgm.) of benzedrine sulfate used in some of the present experiments do exert detectable systemic circulatory effects, it is doubtful that such effects directly affected work capacity by changes in the muscle circulation, since the muscle circulation does not normally appear to be a limiting factor in voluntary muscular fatigue. Whether or not circulatory changes in the region of the central synapses involved exert a limiting effect cannot be speculated upon to advantage at present.

#### SUMMARY

1. Doses of 10, 20 or 40 mgm. of benzedrine sulfate inhibit the production of voluntary muscular fatigue.
2. Doses of 10, 20 or 40 mgm. of benzedrine sulfate may abolish essentially complete voluntary muscular fatigue maintained by repeated but static work trials.
3. The effect may be exerted with respect to increase in extent of each contraction, or in number of contractions, or both.
4. The effect is related fairly well in degree and time with the patellar-reflex activity effects of benzedrine sulfate.
5. The effect is probably primarily related to an action directly on the central nervous system.
6. The effect with benzedrine is more marked than can be observed with ten times as great a dosage of caffeine under the same conditions.

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# OBSERVATIONS ON HEMORRHAGIC HYPOTENSION AND HEMORRHAGIC SHOCK<sup>1</sup>

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During the past year we have restudied the dynamics of the circulation during and after regulated hemorrhage and after reinfusion of the blood withdrawn in the hope that an objective analysis of results would crystallize answers to a number of moot questions. Our primary interests consisted in determining whether hemorrhage *per se* can induce shock and, if so, the conditions for its production, the possibility of standardizing the procedure, the criteria for its recognition and the nature of the eventual failure. Our study strongly suggests that some accepted notions as to the mechanisms concerned in the progression and irreversibility of the circulatory state we call shock may need to be amended.

**ANESTHESIA.** Anesthesia is essential for such dynamic studies. Ether and other volatile anesthetics have not proved satisfactory, because their uneven concentration in the blood and respiratory passages causes humoral and reflex actions which complicate and confuse the circulatory reactions due to hemorrhage alone.

Most of our dogs were given a preliminary subcutaneous injection of morphine sulfate (ca, 3 mgm./kilo) and just enough sodium barbital intravenously (ca, 175–200 mgm./kilo) to complete the anesthesia. In a few experiments sodium amytal<sup>2</sup> (ca, 40 mgm./kilo) was used. The reasons for our choice of barbiturates have been discussed recently (1). However, as further checks, five experiments were carried out under chloralose anesthesia (ca, 7.5 mgm./kilo). We have discovered no essential differences in the dynamic reactions aside from the fact that the heart rate is generally—not always—slower when chloralose is used, and, consequently, the primary cardiac acceleration during hemorrhage is greater.

**METHODS.** Our standard technical procedure involved optical registration of central arterial pressures from the innominate artery and of central venous pressures from the superior vena cava by high frequency manometers of the Gregg type. In addition, intrathoracic pressure was recorded by a segment capsule which was connected to a trocar inserted through the chest wall into the mediastinal space. All recorders were calibrated repeatedly with respect to individual base lines.

In some experiments we made hematocrit readings or noted the appearance of an exteriorized loop of gut. In a number of experiments, one or both vagus

<sup>1</sup> This investigation was supported by a grant from the Commonwealth Fund.

<sup>2</sup> We are indebted to Eli Lilly Co., Indianapolis, for the sodium amytal.

nerves were divided early during the experiment for testing of neuropressor reactions. Operative procedures were always kept at a minimum.

**PROCEDURE.** Thirty-five experiments were satisfactory in all respects, but corroborative information was derived from many others. Blood was generally withdrawn from the femoral artery into a solution of heparin, but in a few cases the whole animal was heparinized.<sup>3</sup>

The plan of our experimentation involved reduction of pressures to low levels by bleeding, maintenance of such levels, as nearly as possible, for various intervals, and eventually reinfusion of the whole quantity of blood withdrawn. To achieve a posthemorrhagic hypotension, various expedients previously suggested by others were tried. In general, three methods are useful for particular phases of such studies:

1. *Stepwise bleeding*, i.e., successive withdrawals of blood volumes, each equal to about one per cent body weight, a reasonable time for recovery being allowed between bleedings. The method is useful in assessing the compensatory mechanisms called into action, but proved less suitable for our purpose because it is difficult to judge the last critical volume that may be withdrawn without jeopardizing the life of the animal.

2. *Continuous slow hemorrhages* (ca, 3-10 cc. per min.) over a period of hours until pressures reach the low level desired. This probably most nearly resembles natural methods for depletion of blood volume, but has the disadvantage that experiments are unduly prolonged and that the intensity of the generalized ischemia is difficult to evaluate on a pressure-time basis.

3. Combination of an initial large, rapid hemorrhage and, after a period of recovery, continuous slow hemorrhages until the desired level of pressure is reached; but bleeding a little more whenever pressure rises above the desired level or reinfusing small amounts when danger threatens. This method has several practical advantages: 1. The duration of an experiment can be materially shortened and, as far as we have been able to judge, without modifying the course of events. 2. It is less difficult to hold pressures at a chosen level for a definite time, hence the degree of generalized ischemia can be estimated more accurately.

**RESULTS.** Results from employment of the last two methods of regulated bleeding may first be briefly analyzed. Segments A to C of figure 1 illustrate the effects of a large and rapid hemorrhage (2 per cent body weight in 2 min.) on the arterial pressure pulses of a dog under sodium amytal anesthesia. Segment D illustrates the recovery attained four minutes later. As a result of the hemorrhage, the heart rate increased almost at once from 160 to 220 per minute, and pressures fell from 153/118 in A to 106/82 in C, recovering to 132/111 mm. Hg in D. Such pressure readings, however, conceal significant differences in the cyclic changes of pressure indicated by the pressure pulses. The central pressure contour in segment A is characteristically normal and need not be described again. The pulse immediately following a large sudden hemorrhage

<sup>3</sup> We are indebted to Dr. L. Klein of Hoffman La Roche, Inc., Nutley, N. J., for the generous supply of Liquaemin which proved highly satisfactory for intravenous injections.

(segment C) displays a large initial spike and a second broader peak during midsystole. Sometimes the initial spike is much larger than in this illustration. The question arises, where systolic pressure should be measured. Since the accentuated spike is due to a momentary overshooting of pressure in the lax arterial system and the midsystolic summit accords with the pressure maximum in the left ventricle, the latter is the more reasonable index. It may be added parenthetically, however, that such spikes may correspond more nearly to auscultatory readings of systolic pressure. If so, sphygmomanometric readings taken clinically would give a false impression of the true systolic pressure. Following a deep incisura and an accentuated after-vibration, the diastolic pressure is nearly horizontal, indicating that no significant "run-off" from the aorta occurs during diastole. This means that tissues receive essentially a periodic systolic flow of blood.

Recovery in the pulse form as well as pressure levels occurs quickly as the aorta is again filled with blood. This is not due to further increase in heart rate; indeed, the rate is slightly slower in segment D than in C (172/min. *vs.* 220/min.). Improved systolic discharge, reduction in capacity of the aorta or increased peripheral resistance may all be concerned. Such arterial records do not permit conclusions as to which, if any, is prepotent. However, we can say that vasoconstriction is far from maximal at this time, for clamping of the carotid arteries caused as great an increase in pressure as it did normally. The right vagus was cut and stimulation of the central end likewise produced a great increase in blood pressure and changes in contour characteristic of an increase in peripheral resistance.

Two other features frequently noted during or after recovery from large hemorrhages are of significance, *viz.*, the development of pronounced Traube-Hering waves and cardiac alternans. The former often caused extreme fluctuations of pressure, but these usually disappeared in 20 to 30 minutes. Ventricular alternans, though less frequent, was by no means uncommon. It is unquestionably brought out by the high heart rates, but often it did not occur until the initial and maximal acceleration had moderated considerably. This suggests that impairment of coronary flow may be quite as important, and that the myocardium does not escape effects of large hemorrhages. Such alternans also was not permanent; presumably some adaptive mechanisms came into action despite a further decline of arterial pressures.

Segment E shows the status after recovery from the initial hemorrhage after a few tests of the animal's pressor responses had been made. These were found to remain normal. The effects of the second progressive and slow hemorrhage are illustrated in segments F to J. As the pressures again decline, the pulse contours do not alter significantly. Details are given in the legends. In segment H, pressures are lower than in segment C, but the spiked feature and flat diastolic characteristic are not shown. As bleeding continued and diastolic pressures became still lower (segments I, J), these features reappeared. The longer persistence of pressure pulses of good form during a slow hemorrhage suggests that the compensatory mechanisms which act *after* a large hemorrhage to

FIG.1

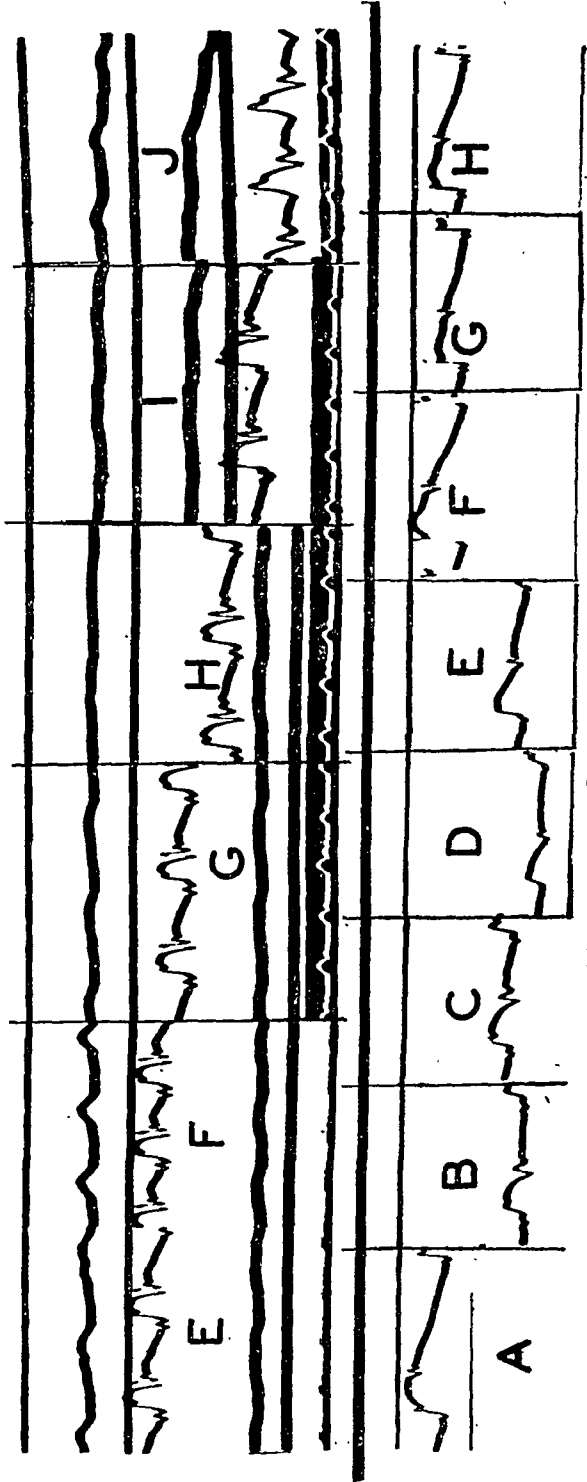
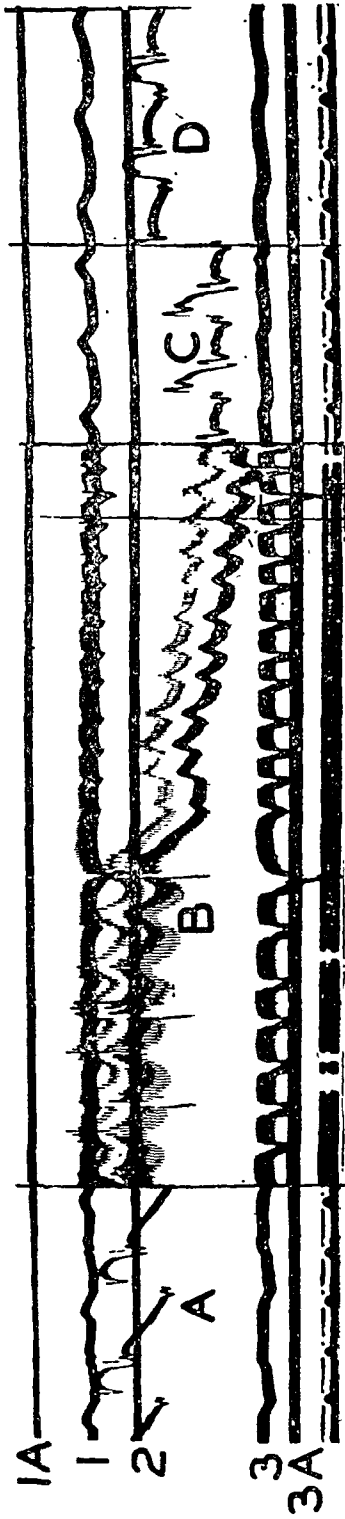


FIG.3

abolish these features (C, D) operate progressively *during* a slower hemorrhage to prevent their appearance.

This interpretation is supported by experiments in which blood pressures were reduced by slow hemorrhages from the start. Thus segments A to F of figure 2 show the effects of a fairly continuous bleeding at an average rate of 3 cc./min. over a period of 2 hours, 28 minutes. With the decline of blood pressure and diminution of the pulse pressures no tendency existed either to spiking or complete flattening of the diastolic limb until segment F. At this time 435 cc., or a quantity of blood equal to 4 per cent body weight, had been withdrawn.

This curve illustrates other aspects of our problem. Shortly after the record of segment F had been taken bleeding was discontinued owing to the fact that the bulb of our projection lantern had burned out. This altered the relative pressure relations in our curves and recalibration was necessary for the other curves reproduced in the second and third rows. Segment G shows the recovery attained 45 minutes later. Since arterial pressures were still 98/72, an additional 30 cc. was slowly withdrawn. The effects of this critical bleeding are shown in segments H and I. In addition to the marked spikes of the pressure curves the heart slowed significantly. This was of sinus origin as shown by the venous pressure curve above. It persisted when both vagus nerves were cut and therefore appeared to be of cardiac origin. As a result of many observations, we are convinced that when the initial acceleration of the heart after large or continuous slow hemorrhages is followed by significant slowing this constitutes a danger signal. As in this animal, respiration soon ceases and the heart becomes extremely slow, as shown in segment J, and the animal dies unless restorative measures are promptly instituted.

Fig. 1. Central venous pressure (1), central arterial pressure (2), intrathoracic pressure (3). Base lines for same (1A), (2A), (3A). Time 0.2 sec. Description in text

	Time	H.R.	B.P.		Time	H.R.	B.P.
A.....	11:53	132	153/118	G.....	12:38	172	111/98
C.....	11:56	220	106/82	H.....	12:45	150	90/72
D.....	11:59	172	132/111	I.....	1:05	150	77/59
E.....	12:32	160	132/112	J.....	1:24	200	56/38
F.....	12:34	200	128/111				

Fig. 3. Central arterial pressures during and after hemorrhage and recovery following infusion.

	Time	H.R.	B.P.		Time	H.R.	B.P.
A.....	10:12	93	132/103	F.....	3:21	120	140/100
B.....	10:40	144	60/45		(after completion of in-		
	(after hem. 3% B.W.)				fusion)		
C.....	11:22	147	68/50	G.....	4:57	120	128/105
	(after hem. 4% B.W.)				(recovery)		
D.....	2:37	132	37/25	H.....	5:10	120	122/102
	(after hem. 5% B.W.)				(recovery)		
E.....	2:52	123	67/48				
	(small inf.)						



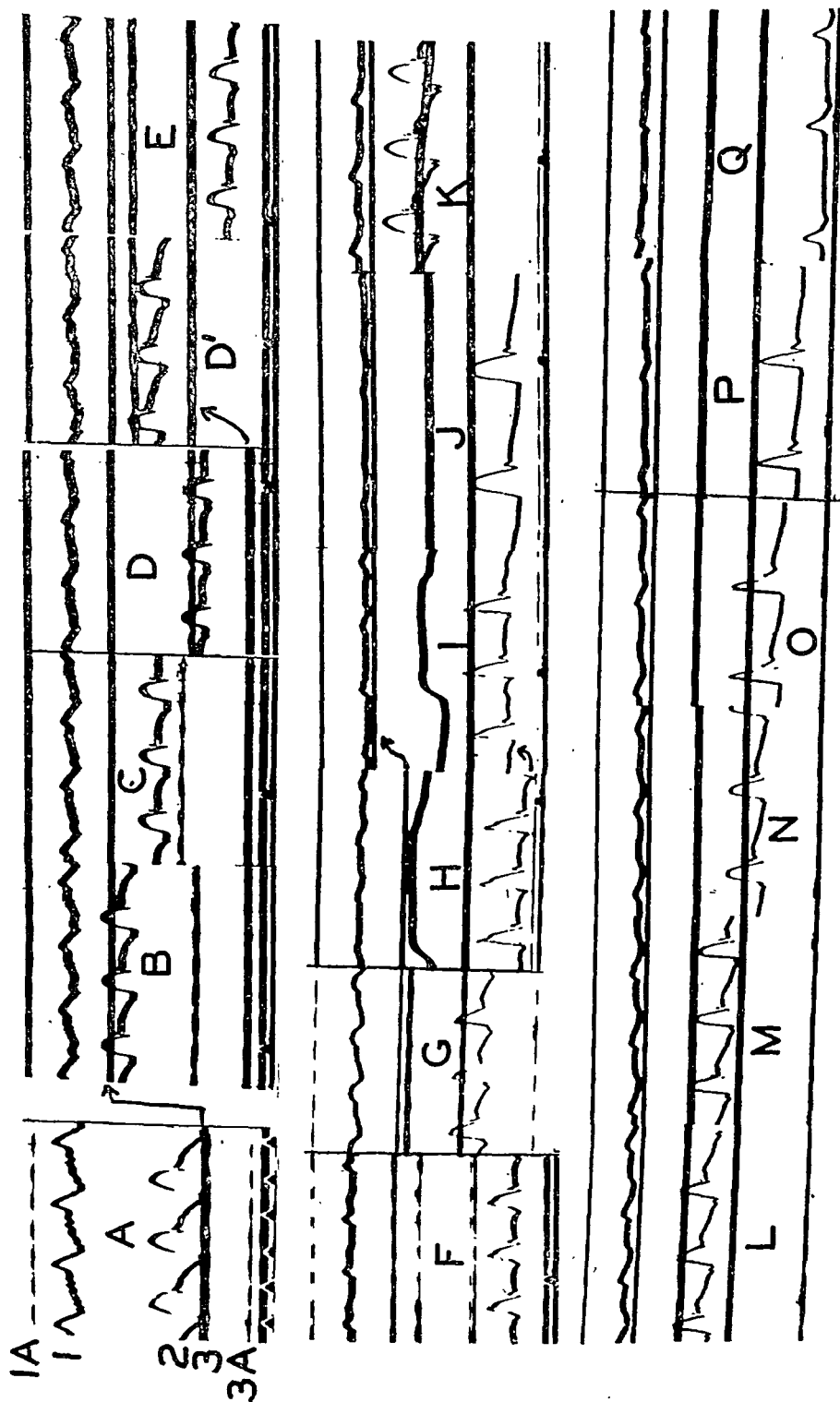


FIG. 2

However, if artificial respiration is given at once and blood is quickly reinfused, the arterial pressures and pulse forms may be restored to normal when all the withdrawn blood has been reinjected. If the pressure pulses of such animals are observed further, however, it is found that some of them maintain arterial pressures and normal pulse forms for three to four hours, whereas others rapidly deteriorate and are dead within 30 minutes to 2 hours. The animals of the first group which nearly died from hemorrhage were rescued through reinfusion of blood; the animals of the second group displaying identical cardiac and arterial pressure changes were only temporarily improved and, we believe, died of hemorrhagic shock. In other words, pronounced posthemorrhagic hypotension may exist for long periods and animals may even die from cardiac or respiratory failure without existence of a circulatory failure that cannot be reversed by substantial injections of blood or plasma. It is important to distinguish death from such *post-hemorrhagic hypotension* from that due to *hemorrhagic shock*, from which dogs cannot be rescued by transfusion.

An example of a favorable response to transfusion is shown in segments D-H of figure 3, the details of which are given in the legends. At the time when segment D was recorded the animal had been bled 5 per cent body weight and had been kept at a mean arterial pressure level of 50 mm. Hg or less over 3½ hours. After a small sustaining infusion of 60 cc. (segment E), the circulation and respiration were maintained for another 35 minutes. Reinfusion of all the withdrawn blood at a critical stage led to restoration of a normal level and form of arterial pressure which was still maintained three hours after infusion (segment H).

An example of an unfavorable ending is illustrated by the remaining curves of figure 2. After reinfusion of the entire volume of withdrawn blood over a period of 46 minutes, the degree of recovery shown in segment K was achieved. Pulse contour and pressure relations were essentially normal; and, moreover, showed no marked changes during the next 30 minutes. However, this state of recovery did not persist. As illustrated by the lower row of segments, pressure progressively declined, the pulse forms deteriorated much as before reinfusion. Toward the end, systolic ejection shortened, the heart again slowed and the animal died within an hour. We again note that estimations of systolic and diastolic pressures alone do not express the degree of circulatory failure, as well as do the pressure pulses.

Fig. 2. Central venous pressure (1); central arterial pressure (2); respiration and arterial base line (3). Base lines for venous pressure (1A), for respiration (3A). Time 0.02 sec. Description in text. Arrows indicate base line shifts between segments.

	Time	H.R.	B.P.		Time	H.R.	B.P.
A.....	10:44	172	180/137	J.....	3:37	105	67/25
D.....	12:12	180	80/62	K.....	4:26	160	135/92
D¹.....	12:35	180	125/100	L.....	5:19	165	108/80
E.....	12:48	188	60/44	M.....	5:30	165	104/70
F.....	2:02	210	61/35	N.....	5:40	136	78/50
G.....	2:49	198	98/72	O.....	5:44	130	75/35
H.....	3:01	198	80/42	P.....	5:45	113	63/30
I.....	3:27	188	70/32	Q.....	5:46	105	27/13

The records of figure 4 illustrate a number of additional dynamic facts in a dog under chloralose anesthesia. Segment A shows the marked sinus arrhythmia which resembles that of unanesthetized dogs at rest. It illustrates admirably the impossibility of assigning definite numerical values to heart rates or to systolic and diastolic pressures. When such control values are reported by investigators who used unanesthetized animals these must either be very approximate or their animals were not in a basal, resting state when experiments were begun.

The effects of an initial large hemorrhage (2 per cent body weight) and recovery therefrom are illustrated in segments B and C. In the latter, slowing and some sinus arrhythmia redeveloped. Such slowing while pressures are still good must not be confused with the dangerous slowing when pressures are extremely low. The consecutive effects of a subsequent slow continuous hemorrhage are indicated in a few selected segments, D, E, F, G, H. Details are given in the legends. Notice that beginning with segment G, both the arterial pressure pulse and base line were moved up. The development of the typical spiked curves and flat diastolic limbs need not be redescribed. It is obvious that development of these dynamic changes was not affected by a change of anesthetic.

At H, the animal had been at a mean pressure of about 40 mm. approximately 30 minutes. Although the animal was breathing well and the heart had not started to slow, a sustaining infusion of 40 cc. blood was deemed advisable because pressures had been at 48/25 mm. Hg for some time. At this time, central vagus stimulation caused a definite and large increase of pressure and intensified the peaked character of the pressure pulse (not illustrated). The animal maintained its respiration with a gradual decline of pressures during the next two hours. At the end of that time, marked slowing of the respiration and of the heart, shown in segment J, supervened. Reinfusion was therefore started promptly, first at a rapid and then at a slower rate. The end result is shown in segment K. Pressure relations were restored to normal ranges (135/95) but only a fair restoration in the form of the pressure pulse was achieved. Segment L shows a curve recorded 10 minutes later. Some lowering of pressures, cardiac acceleration and a further abbreviation of the systolic ejection period were conspicuous changes. Pressures were maintained fairly well for another 30 minutes, then progressive cardiac slowing supervened (segment M) and the animal died. Autopsy revealed no coronary embolism or pulmonary edema.

As a result of many similar observations we feel justified in the conclusions that a state of irreversible circulatory failure is demonstrated when, after a period of marked hypotension induced by hemorrhage, arterial pressures and pulse forms fail to be restored more than temporarily by reinjection of the blood previously withdrawn. In other words, hemorrhagic shock exists. Obviously, also, the ultimate failure occurs despite an adequate circulatory volume in the cardiovascular system. The factor which fails resides in the cardiovascular system, not in the blood volume available.

Comparison of experiments such as are illustrated in figures 2 and 4 suggests a possible difference in the mode of failure. In the former, circulatory failure after reinfusion occurs more gradually, venous pressures rise and the slowing of the heart is a terminal feature. In the latter and less numerous instances, pressures are maintained for an hour or more, than quite suddenly the heart slows, arterial pressures decline, and respiration ceases.

*Determinants of circulatory failure. The problem of standardization.* Our experience emphatically suggests that the development of irreversible circulatory failure after hemorrhage is not determined by the percentage volume of blood lost. Some animals develop shock after withdrawal of a blood volume equal to 3 per cent body weight or much less if extensive operative procedures are carried out at the same time; whereas, others can be restored to normal after a volume equal to 5.0 to 5.6 per cent body weight has been withdrawn. It is true that animals may die as a result of such large hemorrhages but since they can be revived by prompt substantial infusions of their own blood, such death cannot be attributed to shock.

All our evidence suggests that the degree and duration of post-hemorrhagic hypotension are of dominant importance. Our initial experience coincided with that of others (for review *cf.* 1), *viz.*, that many dogs can be kept at pressures between 50–60 mm. Hg for 3 or 4 hours and will recover through subsequent infusion. In order to produce a state which cannot be improved more than temporarily by substantial infusions, arterial pressures must be really low for an effective interval; indeed, generally it must be so low that the circulation is barely maintained and failure of the heart and respiration are threatened.

In order to gain some idea as to the minimal effective intensity and duration of hypotension which causes shock we submitted different dogs to an initial period of moderate hypertension followed by a second period of extreme hypertension. The following procedure was developed. A dog was bled rapidly (ca. 50 cc./min.) up to 2 or 3 per cent of his body weight, but in no event was mean arterial pressure allowed to fall below 60 mm. Hg. After a recovery period of at least 15 minutes, the animal was bled slowly (6–20 cc. per min.) until arterial pressure had been reduced to about 40 mm. If the pressures rose above 60 mm. the dog was cautiously bled a little more. In this way an *initial period* of moderate hypotension (averaging 50 mm.) was maintained for an hour or more. Then the animal was again bled slowly (ca. 6–10 cc./min.) until mean pressures were reduced to about 30 mm., cautiously bleeding a little more whenever pressures approached a 40 mm. level, or infusing a little blood if the heart began to slow or respiration threatened to fail. In this way an *extreme hypotension* at a level of approximately 30 mm. mean pressure was produced.

Our results which are analyzed in detail elsewhere (2) indicate that an initial period of *moderate* hypotension, lasting 90 minutes, followed by a period of *extreme* hypotension for 45 minutes always resulted in irreversible failure. These durations exceed the minimal requirement. Similar periods of moderate and extreme hypotension of 60 and 30 minutes, respectively, were adequate in

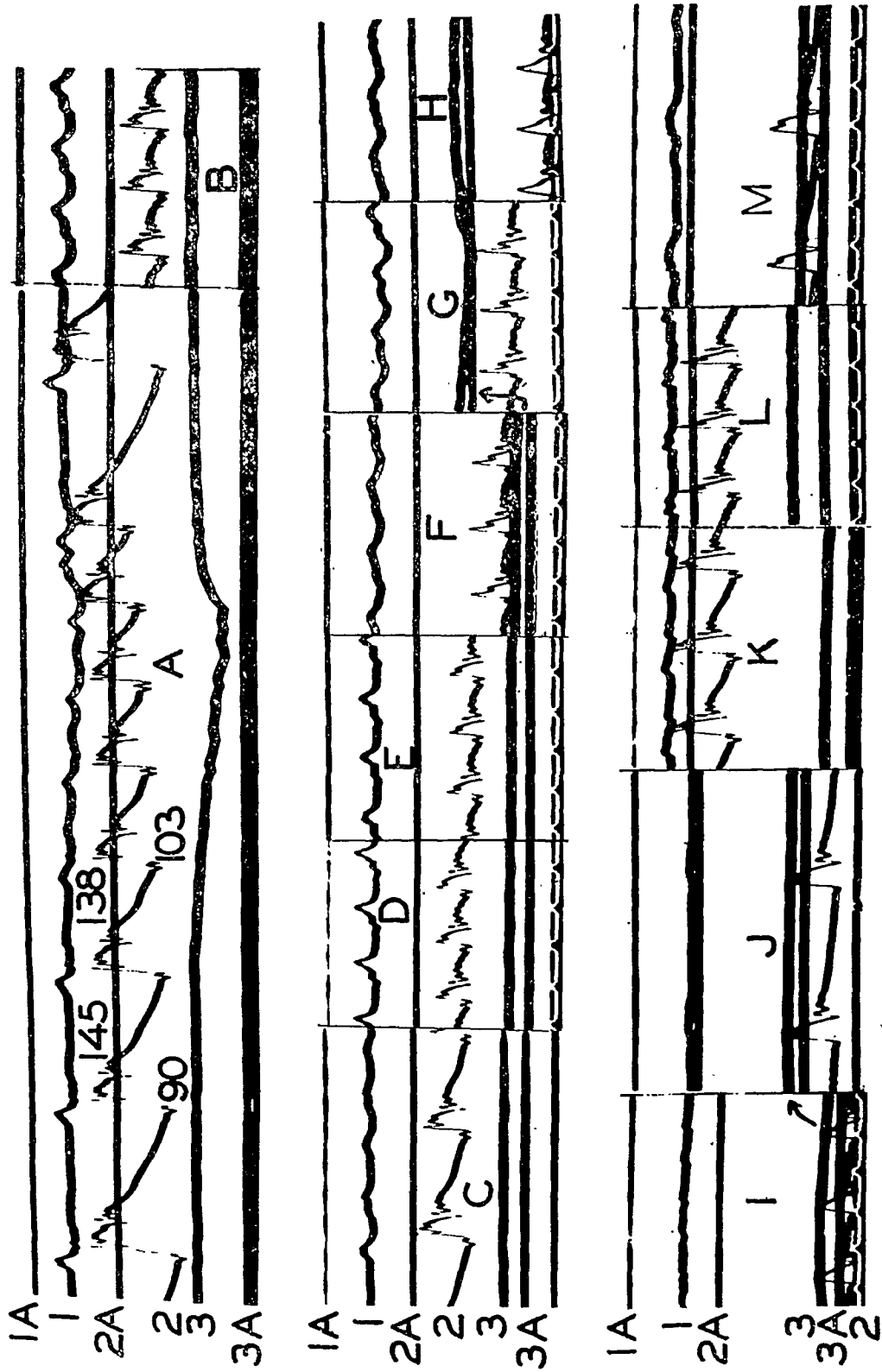


FIG. 4

some animals, but not in the majority. The minimal affective intervals that may prove useful in developing a standardized technique for producing hemorrhagic shock lie somewhere between these ranges.

*Correlations between dynamic criteria of shock and pathological changes in the viscera.* Autopsies were performed in over 90 per cent of our animals. In control animals that died quickly as a direct result of large rapid hemorrhages, pallor of the stomach, gut, mesentery and omentum was a conspicuous feature. The mucosal surfaces were pale and either dry or slightly moist. The spleen was small, hard, firm and rough. Other organs showed no conspicuous changes.

Animals that survived a period of low pressure and were restored by blood infusion for several hours were killed by asphyxia. As a rule, they showed no differences from normal dogs; a few showed scattered petechial hemorrhages in heart and intestinal mucosa, but on the whole the mucosa was normal or very slightly swollen.

Animals in which blood infusion after prolonged hemorrhagic hypotension was only of temporary benefit and which died from progressive circulatory failure usually displayed quite a different pathological picture in the upper intestines. Upon opening the abdomen the mesenteric vessels were not particularly engorged, the liver appeared normal and the spleen was variable, sometimes large and congested, occasionally normal, but generally small and shrunken. The peritoneal cavity contained small amounts of serous fluid but ascites could not be said to exist. On opening the jejunum and ileum, striking pathological changes were generally revealed. In the milder cases, the lumen always contained abundant fluid, heavily tinged with dark bile. The mucosa was swollen and congested and sometimes petechial hemorrhages were noted. In severe cases, the lumen contained considerable quantities of bloody fluid which was signaled in several experiments by passage of bloody stools or staining of a rectal thermometer before death. The mucosa was thick, edematous and congested and contained many bleeding areas. In a few cases, longitudinal striae of congested vessels were noted. With a single exception, in which the gastric mucosa was chiefly involved, the congestive and hemorrhagic condition stopped abruptly in the region of the duodenal bulb; the gastric mucosa appeared pale by contrast. Nor was the mucosa of the ileum or large intestine involved.

Fig. 4. Central venous pressure (1), central arterial pressure (2), intrathoracic pressure (3). Base lines for same, (1A), (2A), (3A).

	Time	H.R.	B.P.		Time	H.R.	B.P.
A.....	10:43—see record			H.....	12:05	190	48/25
B.....	10:55	194	112/89	I.....	1:35	150	45/25
C.....	11:10	112	130/89	J.....	2:14	75	52/20
					(arterial curve and base line raised		
D.....	11:21	214	106/90	K.....	2:46	144	135/95
E.....	11:31	198	95/80	L.....	2:56	182	118/98
F.....	11:43	182	73/55	M.....	3:13	91	60/30
G.....	12:00	185	68/57				

Examination of other organs occasionally revealed scattered petechial hemorrhages on the cardiac and serous surfaces; but these were inconstant. Gross evidence of pulmonary edema never existed. With two exceptions, pericardial fluid was not increased. In short, the pathological changes which correlated with the dynamic criteria were limited fairly strictly to the duodenum and jejunum. They were practically the same as those described by Erlanger and Gasser (3) in shock produced by various procedures.

The question naturally arose whether the mucosal changes preceded or followed reinfusion of blood. To study this problem, a loop of upper intestine was exteriorized in three animals, opened and observed during the whole experiment. The mucosa became more pale during the course of hemorrhages, but while pressures were low cyanosis, congestion and edema developed in the exposed mucosa surface. However, since newly opened sections of gut appeared normal, these effects were apparently caused by exposure rather than hypotension. In three other animals, a duodenal loop was exteriorized *at the end* of a low pressure period regarded as sufficient to cause irreversible circulatory failure, but before reinfusion of blood. The lumen contained fair amounts of secretions and in two experiments a liberal secretion from the pancreatic papillae was observed. The mucosa did not appear congested or swollen in two of these animals, but slight congestion and thickening of the mucosa was present in the other. With injection of blood the mucosa rapidly became darker and more swollen and began to bleed. As circulatory failure developed the luminal contents became bloody. In one animal bloody fluid from other regions was periodically expelled from the opened gut by peristaltic contractions.

The correlation of these pathologic and circulatory changes is interesting, but their meaning is not entirely clear. For example, it remains an enigma why following generalized reduction in blood flow these striking changes are localized so strictly in the upper gut. Apparently, the vessels of this region are particularly vulnerable so that a period of effective hypotension causes some change in the minute vessels of the mucosa and they become intensely congested or even rupture with reinfusion of blood. That such changes are the result rather than the cause of the initial hypotension cannot be argued in this case. That they account wholly for the subsequent circulatory failure after restoration of blood to the circulation must not be inferred too hastily.

Admittedly, the congested bleeding mucosa demobilizes large quantities of blood and additional volumes of fluid are lost into the lumen, but such gross observations do not prove that the effective circulating volume is diminished sufficiently to explain the progressive failure which supervenes.

*Venous pressures during post-hemorrhagic hypotension and hemorrhagic shock.*

The conception that decreased venous return causes a reduction of effective venous pressure and that the latter is responsible for decreased systolic discharge is the keystone of all modern conceptions of shock. This postulate, originated by Y. Henderson (4), has been supported by investigations of one of us (5). However, it could be established in a more crucial manner and it is not a necessary corollary that irreversible circulatory failure only results when

venous pressures have fallen so far that ventricular filling is below a critical level. (For recent review, see Wiggers (1).)

Accurate measurement of venous pressure during the course of a long experiment is not the simple procedure that it is generally thought to be. The following facts need to be kept in mind:

1. We must record central venous pressures within the thorax, not peripheral venous pressures which are subject to local changes in flow.

2. When central venous pressures are recorded by sensitive optical manometers from a sound introduced into the right auricle or superior vena cava, it is found that such pressures are far from constant. A calibrated record is shown in figure 5-III. In such curves the cyclic variations during the short expiratory apnea can vary as much as 135 mm.  $H_2O$ , and during inspiration these values are 60 to 180 mm.  $H_2O$  lower.

3. The mean of the cardiac fluctuations shown in figure 5-III would probably be an adequate index of average filling pressures under normal conditions. Unfortunately, a low-frequency water manometer (damped or undamped) only approximates such readings, owing to the complicated interferences of cardiac and respiratory variations.

4. Since it is time-consuming to enlarge planimeter and integrate optical records such as are shown in figure 5-III, it is more practical to measure these curves at some constant point at all times. The dilemma arises as to which point to choose. Previously, one of us (6) had suggested the pressure value at point X because it represents the pressure under which ventricular filling begins. However, comparison of mean integrated values and pressures at other moments of the cycle, such as X-Y, Z in figure 5-III in several of our experiments, indicated that the pressure at the extreme end of diastole (Z) showed the best correlation. Measurement at this telediastolic point (Z) can also be defended on the grounds that it takes into account the varying vigor of atrial contraction during the course of hemorrhage and shock (*cf.* upper curves of figs. 1, 2, 3) and represents the stretching pressures in the common atrial and ventricular cavities at the very end of filling. We therefore chose this point of measurement (Z) in all of our curves in comparison of recorded venous pressures.

5. Such central venous pressures are recorded by manometers which are balanced against a constant atmospheric pressure; but within the atrium and large veins they are balanced against varying sub-atmospheric intrathoracic pressures. Records of right ventricular pressure show that these variations in intrathoracic pressure are communicated through the right ventricular as well as the atrial walls (7, 8). Therefore, in order to obtain an idea of pressures that are effective in filling the ventricles within the closed chest, it is important, as Y. Henderson and Barringer (9) first emphasized, to measure the algebraic difference between intrathoracic and actual intravenous pressures, *i.e.*, the *effective venous pressure*.

6. Since the tonus of respiratory muscles or mechanical factors may conceivably change intrathoracic pressure during prolonged experiments, it is necessary to measure each pressure separately—not differentially, as was done



by one of us (5)—in order to determine whether changes in effective venous pressure are due to alterations in intrathoracic or actual venous pressures. In twelve experiments such measurements were made, as far as possible, during the period of brief expiratory apnea and, of course, always at synchronous times.

**RESULTS.** Changes in recorded venous pressures are shown in the reduced segments of original optical records in figures 1, 2 and 3. The measured tele-diastolic or Z-pressures, together with intrathoracic pressures and calculated effective venous pressures, are plotted in curves of figure 5. In these, the data indicated by Roman numerals (I, II, IV) correspond to data obtained from records of figures 1, 2 and 4 respectively, and the letters to the segments of records in these figures.

The plots of figure 5, I show 1, a distinct but temporary fall in recorded and effective venous pressures (lower curve) during a rapid loss of blood (A-B); 2, a prompt recovery (C); 3, a progressive decline during the second lower hemorrhage (E-I), and 4, a fair correspondence between changes in recorded and effective pressures, because intrathoracic pressure (IP) changed gradually and not extensively. The latter was an uncommon, not the usual result, as subsequent curves will show.

The plot of figure 5, II shows that the intrathoracic pressures (IP), measured during expiratory apnea, changed considerably during the course of the experiment, and that these changes materially altered the calculated effective venous pressures (EVP); 2, that as a result of a slow hemorrhage (A-E), effective venous pressure declined from 78 to 32 mm. H<sub>2</sub>O; 3, that the magnitude of the decrease was greater than that of the recorded venous pressure, because intrathoracic pressure became less negative; 4, that, consonant with current opinion, the decline of blood pressure and deterioration of pressure pulse contours shown in segments B-E of figure 2 are related to the fall in effective venous pressures; 5, that toward the end of a slow hemorrhage and for approximately an hour after its cessation (E-F), recorded venous pressure (VP) and calculated effective venous pressures (IVP) were approximately restored to normal values, while arterial pressures continued to fall and the form of the pressure pulses deteriorated; 6, that reinfusion of all the blood withdrawn by previous hemorrhage (J-K), elevated the recorded but not the effective venous pressure; 7, that the arterial pressure again fell and the pressure curves began to deteriorate (cf. fig. 2, K-L) despite the fact that recorded and effective venous pressure remained at normal levels for nearly an hour; 8, that the final collapse of arterial pressure pulses (L-P) was associated with slight decline of recorded and effective venous pressures, but 9, that when the circulation was very low (Q), as well as immediately after death, recorded and effective venous pressures equaled levels found at the start of the experiment. The trend of venous pressures shown in this plot was typical of those in eight other experiments.

The curves of figure 5, IV illustrate venous pressure changes characteristic of four other experiments. This plot shows 1, that, as anticipated, actual and effective venous pressures fell markedly during the initial large hemorrhage (A, B), and partially recovered therefrom shortly after hemorrhage had ceased

(C); 2, that a second but slower hemorrhage (C, G) reduced recorded and effective venous pressures fairly permanently,—while arterial pressures remained

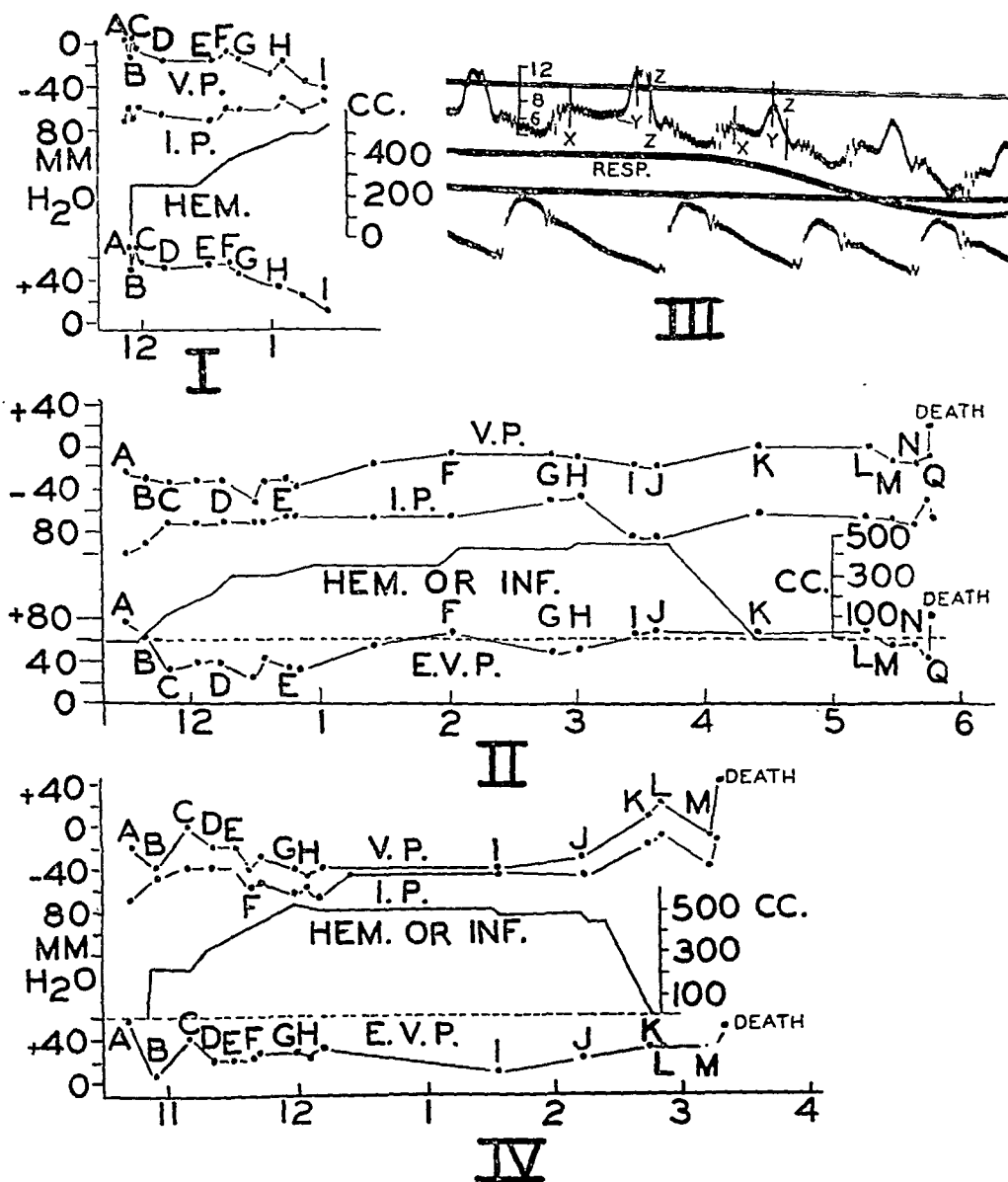


Fig. 5. I, II, IV, plots of actual venous pressure (V.P.), intrathoracic pressure (I.P.), and effective venous pressures (E.V.P.) plotted in relation to hemorrhages and infusion. From curves represented by segments of records in figures 1, 2 and 4. III, original tracing of venous, intrathoracic and subclavian pressures showing variations of venous pressures. Description in text.

low for the next two hours, and the pressure pulses stayed abnormal (H, I); 3, that the serious circulatory failure shown in segment J of figure 3 was attended by a slight increase in effective venous pressures between I and J, due to a rise of recorded venous pressure; 4, that reinfusion of the lost blood raised recorded

pressure greatly and effective pressure but slightly (K, L); 5, that the subsequent sudden decline of arterial pressure and slowing of the heart (M) were attended by a reduction in recorded, but no change in effective venous pressures.

*Deductions.* In the first place, withdrawal or reinfusion of blood—particularly if fairly rapid—causes significant changes in intrathoracic pressure, which often make determinations of actual venous pressure deceptive as regards quantitative and even directional trends. As illustrated in figures 5, II, IV, reinfusion of blood—as may be expected—raises intrathoracic pressure, i.e., renders it less negative. However, as these graphs also show, withdrawal of blood often has the same effect. It is tempting to assign this to a reduction in tonus of respiratory muscles during expiration, particularly as Gesell and Moyle (10) recorded a slight lengthening of skeletal muscles during hemorrhage; but we have no direct proof for this.

In the second place, no question exists that recorded and effective central venous pressures drop significantly soon after the start of a large hemorrhage equal to 2 per cent of the body weight or more, and to judge from pressure pulses systolic discharge decreases at once. However, after cessation of such hemorrhages venous pressures rise promptly, often returning to normal.

In the third place, during the period when arterial pressures have been stabilized at low levels by one large and successive slow hemorrhages, the recorded and effective pressures remain low in the minority of animals. In the greater number, illustrated by graphs of figure 5, II, both recorded and effective venous pressures rise, often to normal levels. It seems significant that this recovery is not associated with a similar improvement in arterial pressures or in the form of the arterial pulse curves. Similarly, after all the withdrawn blood has been reinfused following an effective period of low pressure, venous pressures rise and in the majority of animals remain at normal or supernormal levels. Nevertheless, arterial pressures decrease again and the form of the arterial pressure pulses deteriorate. While in some animals ultimate circulatory failure parallels decline in venous pressures, the fact that in a greater number similar failure occurs without such decline makes it apparent that failure can be due to other causes in hemorrhagic shock. Henderson's dictum, "venous pressure is the fulcrum of the circulation," certainly does not hold in this type of hemorrhagic shock.

In the fourth place, regardless of previous trends, recorded and effective venous pressures always increase when the heart begins to slow during terminal stages, apparently because of stasis. This emphasizes again that venous pressures are determined by at least two factors: 1, the volume of venous return, and 2, the volume pumped away by the heart. It is therefore conceivable that the return of venous pressures to or toward normal while arterial pressure declines may be due to the fact that a reduced venous return is nicely counterbalanced by the decreased cardiac output. However, if this be true, primary myocardial depression must be assumed.

*Hematocrit determinations.* These were originally instituted because, in our experiments, it was necessary to flush various cannulae of optical manometers periodically in order to assure ourselves of their complete patency. During the

course of long experiments considerable quantities of heparinized Ringer's solution were thus introduced which might be expected to increase the natural hemodilution following hemorrhage. Consequently, in nine experiments we attempted to gauge the extent of such dilution and its effect on dynamic changes. To our surprise we found no unusual decrease in hematocrit readings during the period of post-hemorrhagic hypotension, and a slight increase in cell/plasma ratios after reinfusion. Typical results are given in abridged table 1.

TABLE 1

SAMPLE NO.	TIME	HEMATOCRIT	B.P.	REMARKS
Dog K-17, 10 kilos, morphine, sodium barbital				
1	9:55	43.5	120	Control
2	11:50	30.8	60	13 min. after total 475 cc. hemorrhage
3	1:20	48.2	80	After 90 minutes' hypotension and reinfusion
4	2:00	47	58	
5	3:25	44	45	Natural circulatory failure
6	4:12	44.4	20	Death followed
Dog K-30, 10 kilos, morphine, sodium barbital				
1	11:14	45.5	135	Control
2	11:45	39.6	50	After 90 minutes of moderate hypotension
3	12:37	41.0	35	
4	1:28	42.3	40	
5	2:22	45.9	35	
6	3:27	48.4	60	After reinfusion
7	4:25	46.2	35	Decline and death
Dog K-20, 11 kilos, morphine, sodium barbital				
1	10:31	47.3	155	Control
2	10:58	39.4	60	26 min. after 550 cc. hem.
3	11:27	36.9	40	24 min. after 40 cc. hem.
4	12:05	39.9	30	33 min. after reinfusion 500 cc.
5	12:55	41.5	30	43 min. after reinfusion 550 cc.
6	1:56	52.0	118	28 min. after reinfusion all blood
7	2:47	51.9	120	
8	3:21	51.9	120	2 hrs. after infusion Apparent recovery, no gut changes, etc.

Since the processes leading to shock were apparently operating during the post-hemorrhagic hypotensive period while hemodilution existed, our experiments lend little support to the idea that development of shock is necessarily related to hemoconcentration.

*Plasmapheresis experiments.* In order to test further whether shock developed more quickly and certainly when loss of blood volume was accompanied by hemoconcentration, six experiments were performed in which the blood withdrawn

was centrifuged and the red corpuscles returned according to standard procedures. Our results showed that after each reinjection of a substantial volume of corpuscles, the cell/plasma ratios and presumably the blood viscosity and peripheral resistance increased. Such injections made it more difficult to reduce the arterial pressures and hence to induce a state of shock by withdrawal of equivalent circulating volumes. It was our impression that hypotension rather than hypovolemia *per se* is the dominant factor in shock following hemorrhage. While we hesitate to draw general conclusions with regard to the contributing rôle that spontaneously developing hemoconcentration may play in development of other types of shock, it was our impression that artificial hemoconcentration retards rather than hastens the development of hemorrhagic shock.

DISCUSSION. *The experimental criteria of shock.* While the syndrome now called "shock" probably involves disorganization of systems other than the circulation, a progressive circulatory failure which becomes irreversible without treatment is unquestionably a dominant feature.

Our experiments indicate that while the state of hypotension which follows severe hemorrhage is not necessarily equivalent to shock, it can, if sufficient in degree and duration, lead to such a state, and apparently as a result of prolonged hypotension rather than hypovolemia *per se*. Our experimental and pathologic evidence suggests that both the peripheral circulation and heart contribute, in different proportion in different animals, to the phenomenon of irreversibility.

Unfortunately, no criterion has yet been discovered by which the existence of such an irreversible state can be determined. Neither changes in arterial or venous pressures nor characteristics of arterial pressure pulses offer any criterion which distinguishes between hemorrhagic hypotension or shock. Irreversible shock develops under states of hemodilution or hemoconcentration. The only reliable criterion seems to be whether or not an animal recovers after substantial infusions of whole blood, and possibly of plasma or serum.

While we recognize the clinical disadvantage of restricting the term to hopeless terminal conditions which cannot be improved more than temporarily by infusions, we feel that such limitation would correlate experimental and pathological criteria, and it would focus greater attention on the nature of irreversible mechanisms and efforts to counteract them. Above all, it would tend to establish among laboratory investigators more rigid criteria for testing agents and measures reputed to have therapeutic value. Maintenance of arterial pressure at low levels for several hours as a result of any procedure is not a sufficient index that irreversible circulatory failure exists. The use of such animals for the assay of various therapeutic agents does not constitute a crucial test. If, on the contrary, such agents prove effective after an animal has failed to respond to substantial infusions, some claim of efficacy is allowable.

#### SUMMARY

By the expedient of regulated hemorrhages, dogs anesthetized with sodium barbital, amytal or chloralosane were kept in a state of severe hypotension for

varying intervals, after which all the withdrawn blood (heparinized) was reinjected. Central arterial, central venous and intrathoracic pressure changes were recorded throughout the experiments.

A continuing state of post-hemorrhagic hypotension is not necessarily equivalent to shock, for in many animals *a*, arterial pressures and pulses were restored to normal for many hours by reinfusion of the withdrawn blood even when such animals were on the verge of cardiac or respiratory failure, and *b*, the viscera showed no pathological changes at autopsy.

If, however, both the intensity and duration of the post-hemorrhagic hypotension were great enough, *hemorrhagic shock* developed for *a*, the condition was only temporarily benefited by generous infusion of blood, and *b*, the duodenal and jejunal mucosa was generally edematous, congested and bleeding with presence of excessive fluid and blood in the lumen. Other organs showed no consistent pathological changes.

While an elusive "resistance factor" interferes with attempts to standardize the procedure for experimental purposes, our results suggest that the greatest hope lies in creation of a preliminary period of moderate hypotension (*ca.* 50 mm.) followed by a shorter period of extreme hypotension (*ca.* 30 mm.). Using such a scheme, the minimum effective durations for these respective stages were found to be less than 90 and 45 minutes and more than 60 and 30 minutes in our trials.

We have discovered no new dynamic criteria which enables us to determine whether a period of post-hemorrhagic hypotension will be followed by failure or recovery on reinfusion. Indeed, hemorrhage equal to 3 to 4 per cent body weight can produce all the changes in arterial pressure pulses seen in most severe shock due to other causes (5). Irreversibility after substantial infusion, admittedly unsatisfactory as a practical guide, is unfortunately the only reliable one at present.

Circulatory failure following reinfusion of all withdrawn blood developed despite an adequate blood volume and regardless of hemodilution or hemoconcentration. Augmented hemoconcentration induced by plasmapheresis had no discoverable accelerating action. The factor which precipitates the irreversible state of hemorrhagic shock resides in the cardiovascular system. Reduction of effective venous pressure did not account for the failure of arterial pressures in the majority of our animals. Since, in the majority, effective venous pressures were at or above normal levels during the post-infusion decline of arterial pressures, the deterioration of arterial pressure pulses must be due to impairment of the heart, changes in the aorta and its elastic branches and/or in the resistance at the periphery of the arterial tree.

The supervention of cardiac alternans during early periods of post-hemorrhagic hypotension, the later tendency toward progressive slowing, the poor response to rapid infusions and the terminal rise of venous pressures suggest operation of a cardiac precipitating factor in some of our animals.

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# CARDIAC AND PERIPHERAL RESISTANCE FACTORS AS DETERMINANTS OF CIRCULATORY FAILURE IN HEMORRHAGIC SHOCK<sup>1</sup>

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In a previous communication (1) we reported that the state of hypotension which follows severe hemorrhage is not necessarily equivalent to that of shock but that the progressive circulatory failure which supervenes after an effective period of such hypotension and the reinfusion of all the withdrawn blood warrants the designation, *hemorrhagic shock*. In some animals such a state developed without a reduction or even with an increase in central venous pressures, which suggested that failure of the myocardium might be a factor.

In a recent review (2) one of us pointed out that a number of investigations strongly indicate that arterioles of certain regions, particularly those of the limbs, salivary glands and spleen, constrict but that this conclusion is by no means unanimous. Since total peripheral resistance (TPR) is determined by numerous regional resistances arranged in parallel ( $1/TPR = 1/R_1 + 1/R_2 + \dots + 1/R_n$ ), changes of resistance in one territory may not give a correct index as to the extent that changes in TPR favor or antagonize the progressive downward course of arterial pressure in shock.

This report attempts 1, to evaluate the state of myocardium and TPR during prolonged post-hemorrhagic hypotension and following reinfusion of all withdrawn blood, and 2, to assess the relative importance of changes in venous pressure, myocardial responses and TPR in the promotion or prevention of irreversible circulatory failure.

**METHODS.** Optical records were made periodically of aortic and right atrial pressures together with volume changes of the ventricles. The methods employed for the latter are described elsewhere (3). In addition, records of mean femoral pressure and changes in ventricular volume were recorded continuously on a slowly moving smoked drum. The optical volume curves were used for evaluating the mechanisms of ejection and filling; the drum records, to estimate changes in systolic discharge and minute output quantitatively. For this purpose, the records were calibrated frequently, while the heart was beating in the cardiometer, by withdrawing or introducing known volumes of air (fig. 1, D). This also established absence of leaks.

Total peripheral resistance was calculated by the formula,

$$TPR = \frac{\text{mean pressure} \times 1332}{\text{cardiac output/sec.}} = \frac{\text{dynes} \cdot \text{sec.}}{\text{cm.}^5}$$

We shall refer to these as absolute units (A.U.).

<sup>1</sup> Supported by a grant from the Commonwealth Fund.



*Critique of methods.* The value of our conclusions depends largely on the accuracy with which qualitative and quantitative changes in ventricular filling and output are determined by cardiometric methods. A brief evaluation of available methods and the precautions that must be exercised is therefore indispensable. Cardiometric registrations introduced by Y. Henderson have proved particularly valuable in analyzing the mechanisms of cardiac filling and emptying when directional changes occur within short periods. Even in such studies, technical errors have occurred and misinterpretations have arisen as Y. Henderson (4) and later Wiggers and Katz (5) have pointed out. For example, the end of ejection and the magnitude of the stroke cannot always be determined precisely without simultaneous records of aortic pressures.

When the ventricular volume curves are used quantitatively in experiments of long duration, errors easily creep in despite meticulous efforts to fix the cardiometer diaphragm with respect to the A-V junction. Whenever the capacity of the ventricles and the circumference of their base alter considerably, as in our experiments, it requires a good deal of judgment and practice to maintain an ideal placement of the heart and a perfect fit of the rubber diaphragm throughout the experiment. When the circumference of the ventricular base decreases after severe hemorrhage, leaks tend to develop; when it gets larger after infusion a mechanical A-V stenosis may occur.

We were able to minimize these difficulties by allowing the open margin of the rubber diaphragm to form a rolling cuff about the ventricular base. This cuff tended to be taken up as the circumference decreased during hemorrhage and to roll up as the heart dilated during infusion. As a rule, the decrease in diameter during bleeding did not cause any leak, as verified by calibration tests. However, when the heart dilated as a result of a rapid reinfusion, a slight stenosis occasionally developed. Fortunately, this was evidenced by an inordinately large atrial wave in the right atrial pressure curve and an altered mode of ventricular filling. Such an extreme effect is illustrated by comparison of curves A and B of figure 1. In curve A, obtained after hemorrhage, atrial pressure decreases with the onset of ventricular filling at Z and the subsequent atrial contraction causes a moderate elevation of atrial pressure (A). In curve B, obtained shortly thereafter, subsequent to a large infusion of blood, the pronounced rise of pressure during atrial contraction is chiefly responsible for ventricular filling; indeed vibrations presumably due to an associated murmur are superimposed on the volume curve. Such curves must obviously be discarded. Fortunately, such tendency to mechanical stenosis disappears quickly. Furthermore, after we recognized the possibility of this artifact, it could generally be avoided, as shown in curve C, by grading the infusion rate to the capacity of the ventricle to move blood forward. However, if volume curves show a more gradual filling rate toward the end of an experiment, the question often needs to be settled whether this was a natural phenomenon or was due to development of A-V constriction as the ventricles progressively dilated. The amplitude of the optical atrial pressure pulse is always a useful guide in such cases. If the pressure wave due to atrial contraction is extremely large, a stenosis effect must

periods. But the manner in which this reduction was accomplished, the changes in TPR which occurred and the effects on arterial pressures and pressure pulses were strikingly different in different experiments.

An analysis of these variations requires a fairly detailed consideration of at least three of our experiments. In order to conserve space, the data have been reduced to three charts (figs. 2, 3, 4), each of which shows plots of the time and extent of bleeding and reinfusion, changes in heart rate, mean arterial pressure, total peripheral resistance in absolute units, cardiac output per minute, together with transcribed tracings of selected ventricular volume curves and

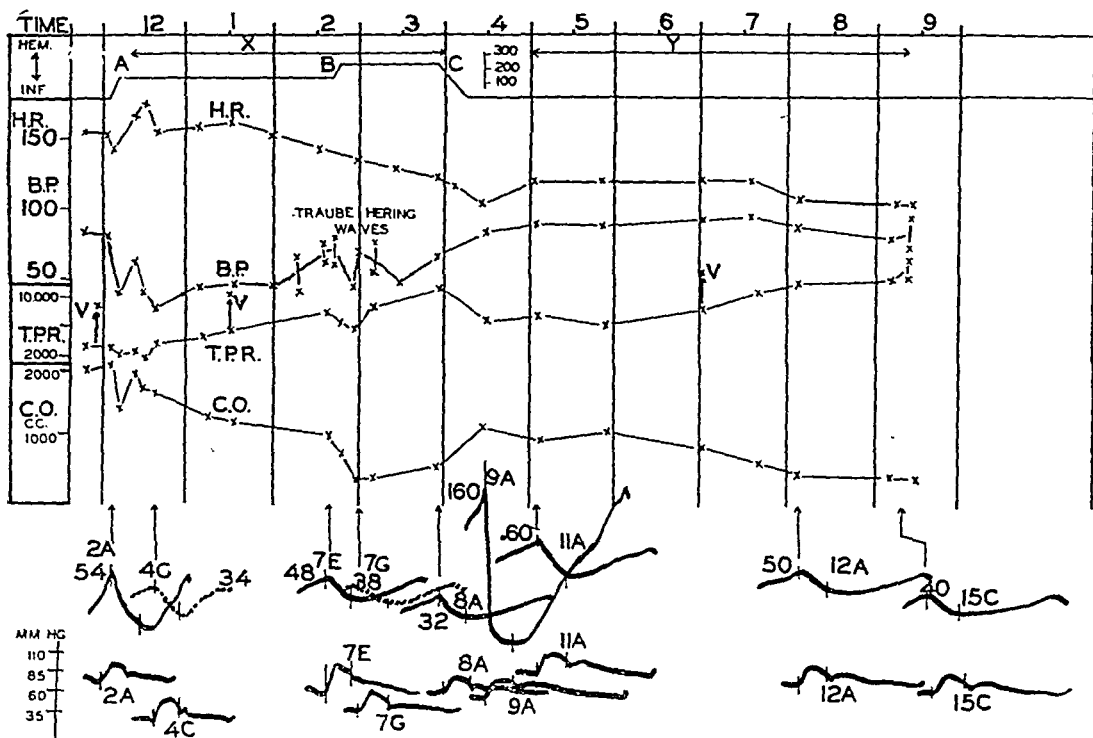


Fig. 2

aortic pressure pulses. Venous pressures are directly inscribed with each volume curve.

Figure 2 illustrates results from a dog in which arterial pressure was kept below 50 mm. Hg for  $1\frac{1}{2}$  hours by a first hemorrhage of 170 cc. (A), and when arterial pressures increased thereafter the animal was bled another 90 cc. (B), but this only reduced pressures to 50–70 mm. Hg for another half hour. The span of the post-hemorrhagic period is indicated by the line, X. While mean pressure was gradually rising, all the withdrawn blood was reinfused at C. Five and a half hours later (ca, 9 p.m.), mean arterial pressure was as high as at 11 a.m., and the heart rate had decreased from 150 to 100 per minute. Venous pressure had not changed significantly at the end. By no easily determinable criterion could shock be said to be impending and we labeled this a

recovery animal. Nevertheless, an analysis of changes in stroke volume, minute output and total peripheral resistance suggests that such a process may have been in progress. It stresses the need for care in making a prognosis on the basis of sustained arterial pressures alone.

During the hypotensive period following the first hemorrhage, we note that cardiac minute output decreased progressively and that this was followed by a further decrease after the second hemorrhage. The volume curves below show a typical decrease in diastolic filling and stroke, the trend corresponding to reduction in venous pressures. Nevertheless, mean arterial pressure and pressure pulses showed considerable recovery due to the progressive increase in total peripheral resistance (TPR). This increase was never maximal, as shown by an additional increase in mean pressure and total peripheral resistance following stimulation of the central left vagus (marked V on TPR curve). In addition, marked Traube-Hering variations occurred just before and after the second hemorrhage. With reinfusion of blood, the heart rate slowed—the customary finding in our laboratory—but the tremendous increase in venous pressure and stroke volume shown in curve 9A served to elevate mean pressure despite a prompt reduction in TPR. At autopsy, the duodenal mucosa appeared congested but not hemorrhagic.

During the post-infusion period (Y), the curves clearly show that arterial pressure was sustained by an increasing TPR, while ventricular filling and stroke volume progressively decreased and venous pressures declined.

Comparison of the volume curves recorded at essentially equal venous pressures, e.g., 2-A, 11-A, 12-A and 4-C, 7-G and 15-C suggest progressive diminution in stroke with respect to comparable venous pressures, but the variations might be within the limits of experimental registration. Certainly, in comparing the reactions of more proximate recordings, e.g., 7E, 7G, 8A, 9A, 11A, 12A and 15C, it could be said that the changes compare qualitatively with those described by Starling (7) in the heart-lung preparation and by Wiggers and Katz (5) for the controlled circulation preparation. In a general way also, we may say that cardiac output during the post-infusion period decreased in part through slowing of the heart and in part through reduced stroke volume consequent to diminishing venous pressures, and that arterial pressure was maintained by compensatory increase in TPR. In other words, an incipient state of circulatory failure was developing after the manner first described by Y. Henderson (8).

A similar course was followed in five other experiments. In two of these, TPR remained increased during the post-infusion period, nevertheless, they terminated fatally. In two other fatal experiments, no significant changes in TPR occurred throughout the experiment, and in one it alternately increased and decreased, leveling a little above normal toward the end. Such results indicate that hemorrhagic shock can occur regardless of whether TPR is high, normal or low.

The experiment charted in figure 3 illustrates a number of different features. To judge from cardiac output data and the good arterial pressure and pulse at

the start, this animal appeared in much better condition initially. However, withdrawal at A of only 180 cc. (1.8 per cent body weight) caused so precarious a fall of blood pressure that 50 cc. was promptly reinfused at B. Following this, arterial pressure was maintained above 50 mm. for about 30 min., and thereafter progressively declined to about 35 mm. Hg, at which level it remained for about

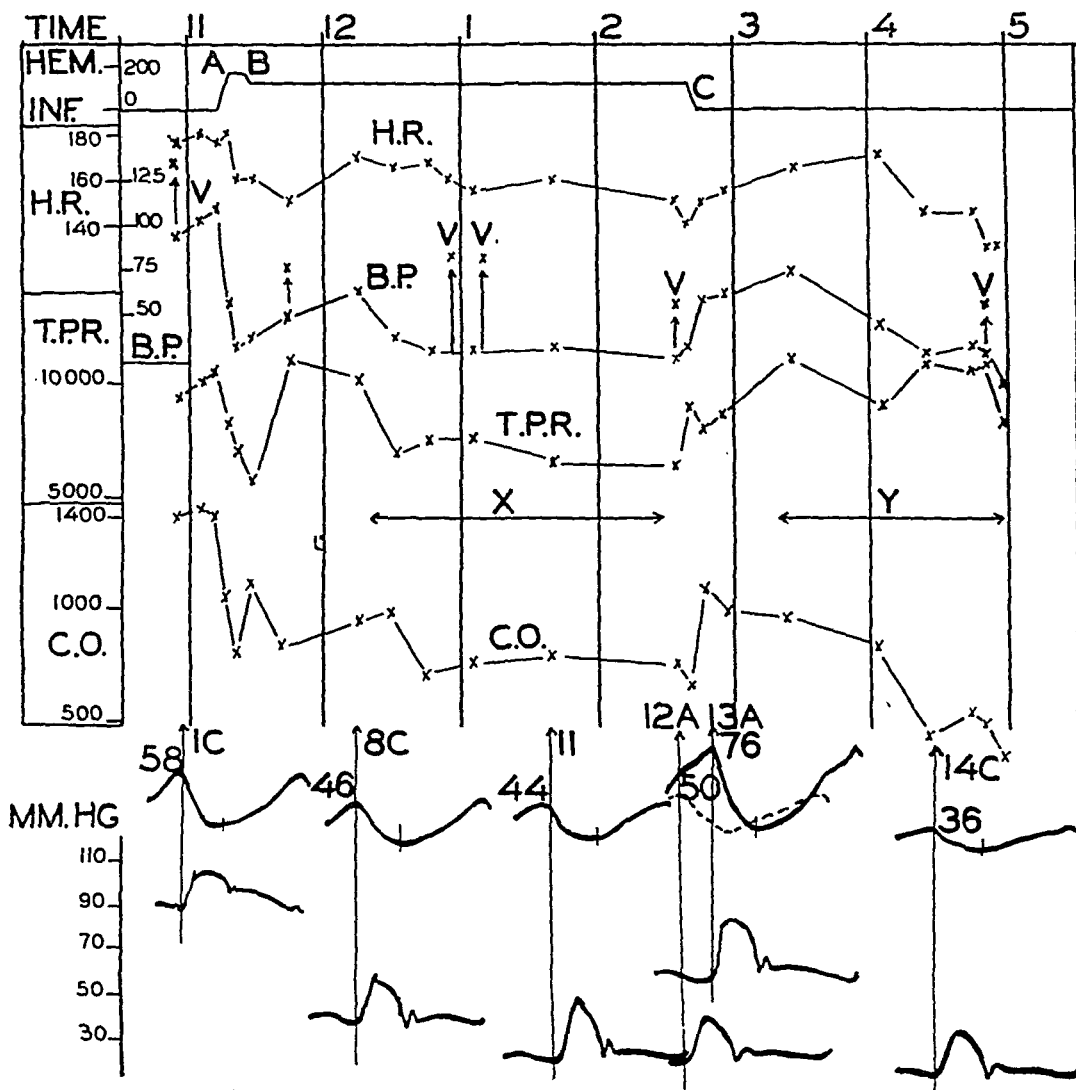


Fig. 3

two hours before reinfusion was started. The effective hypotensive period is indicated by the line X. The directional trends shown in volume curves and pressure pulses drawn below (1C, 8C, 11, 12A) are those anticipated from a decreasing venous pressure, except that the total decrease in venous pressure was not large. The plot of TPR during the post-hemorrhagic period in this experiment shows a progressive reduction which, conjointly with the reduced output (CO), is responsible for the rapid decline of arterial pressure to pre-

carious levels. Contrary to the experiment of figure 2 also, TPR increased after reinfusion of blood, but never above normal during the significant post-infusion period (Y) in which the arterial pressures and pulses deteriorate progressively.

Stimulation of the left vagus at various times (denoted by V) showed at all times a marked increase in pressure, as well as in TPR (not charted) proving that reduction of TPR during the post-hemorrhagic period was not due to the inability of the vasomotor center to react. It must, therefore, be assigned either to failure of the afferent sides of pressor arcs or to some dominant peripheral action. In two other animals, a reduced tolerance to loss of blood was also associated with decrease in TPR, which suggests a possible factor in the variable resistance of animals to hemorrhage.

In this or similar experiments any functional damage inaugurated during the phase of hemorrhagic hypotension could not have been initiated by vasoconstriction. Nevertheless, during the post-infusion period (Y), failure of the circulation progressed much more rapidly than in the previous experiment. At autopsy, the duodenal mucosa showed marked hemorrhagic changes, with presence of free blood in the lumen. A careful study of volume curves, illustrated by a few transcribed curves, again revealed no significant deviation from the mechanism described by Y. Henderson. Since TPR never increased above normal, the progressive decrease in cardiac output, clearly due to decreasing venous pressure, rapidly led to irreversible failure. It is perhaps hazardous to say that the realized reduction in cardiac output never leads to irreversible circulatory failure as long as compensatory increase in TPR occurs, but our experiments strongly suggest that absence or sudden failure of such a peripheral state from any cause is a potent factor in its precipitation.

This mode of failure was not followed in three of our experiments in which TPR remained high and venous pressures stayed above normal until the end, or even manifested a tendency to rise as cardiac output decreased and arterial pressures and pulses deteriorated rapidly. These experiments resembled those reported in a previous paper in which myocardial depression seemed to be suggested. Figure 4 illustrates this type of reaction in a dog that initially appeared highly resistant to loss of blood. As shown in the graphs, it required three successive hemorrhages (A, B, C) of 260, 110 and 90 cc. (total 4.6 per cent body weight) to reduce arterial pressure to about 50 mm. After the last hemorrhage, a hypotensive period less than 50 mm. was obtained and maintained for more than an hour (line X). The various curves of this chart show that previous to the third hemorrhage, the fall of blood pressure was due to a decrease in both cardiac output and TPR. This indicates that certainly in operated animals, a compensatory increase in TPR once induced may subsequently fail, or it may be delayed considerably. Thus, following the last hemorrhage (C), TPR increased progressively while cardiac output declined. The latter was due both to cardiac slowing and decreased stroke volume, shown by comparison of curves 2A, 6A and 8B. However, just previous to the infusion at D, the stroke volume in curve 11C diminished much more despite a slight but unmistakable increase in venous pressure, which in turn might be attributed to the concurrent cardiac slowing.

At D, infusion of blood was started and at E about 100 cc. had entered. As shown in curve 13A venous pressures rose extremely, the cardiac stroke became very large but owing to prompt reduction of peripheral resistance the arterial pressure and pulse were not immediately improved. However, as the action of the heart improved progressively and its rate increased, arterial pressures rose above normal levels and remained elevated for approximately 20 minutes.

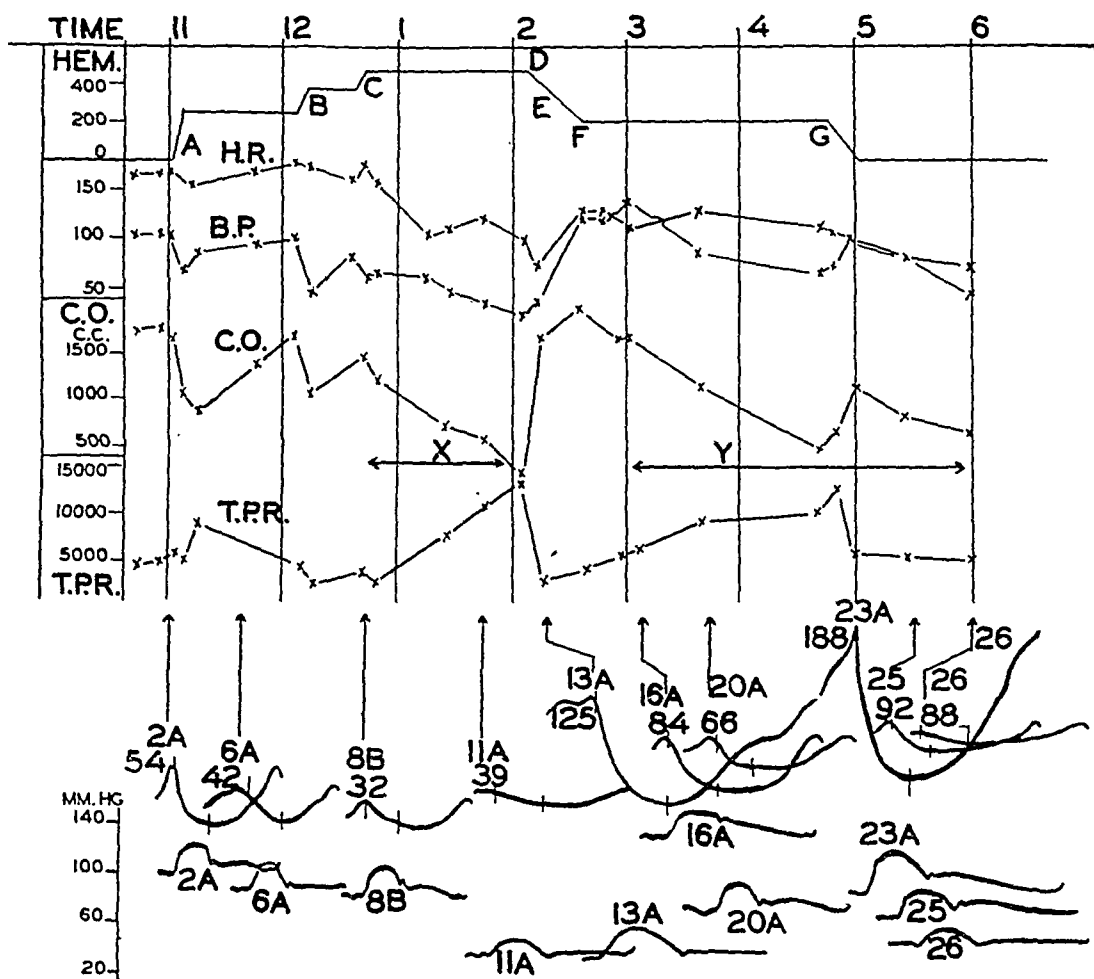


Fig. 4

After 3 p.m., however, arterial pressures and pulses began to deteriorate rapidly despite an augmenting TPR. About 4:45 the remainder of the blood still available (160 cc.) was injected. The improvement in cardiac output, arterial pressures and pulses which followed was very temporary, however, and was followed by a rapid decrease in all during the next hour. At 6 p.m. the heart became very slow and then fibrillated.

While failure to maintain a high TPR was contributory, the rapidly diminishing cardiac output was the chief mechanism that precipitated circulatory failure during the post-transfusion period (Y). But in this case it was not associated

with decline in venous pressure; on the contrary, it was attended by a significant rise until the very end. As a matter of fact, the fortuitous division of the blood for reinfusion so that a considerable volume could be given near the end made manifest a condition that might otherwise have escaped attention.

A careful survey of the volume curves confirms a suspicion already alluded to in other experiments and allows an explanation for the cardiac failure. In comparing volume curves 11A and 13A, we note the improvement in filling and the great increase in stroke volume expected as a result of the rise in venous pressures from 39 to 125 mm. H<sub>2</sub>O which follows the first infusion (D-E); but as venous pressure subsequently decreased to 84 and 66 (curves 16A and 20A), the stroke volume reduced extraordinarily. Comparing these curves with 2A we note that the stroke volume under these pressures was actually less than at 54 mm. H<sub>2</sub>O in 2A. The last reinfusion (G) once more raised venous pressure temporarily to 188 mm. H<sub>2</sub>O with an enormous increase in filling rate and stroke (curve 23); but as venous pressures declined to 92 and 88 mm. H<sub>2</sub>O (curves 25 and 26) the rate of filling became very slow and the stroke exceedingly small. This impairment of filling and ejection was clearly not due to failing venous pressures; on the contrary, it occurred with supernormal pressures. A-V constriction by the rubber dam was excluded. It was not due to an increase in hypothetical myocardial tonus, for the records as well as inspection of the ventricles in the glass cardiometer revealed that they were excessively dilated, particularly on the right side. Consequently the only allowable inference is that a hypodynamic action of the ventricles was responsible for this accumulation of blood and that the rise of intraventricular pressure thus occasioned impeded the diastolic filling (cf. curves 16A, 20A, 25 and 26).

It is true that such ventricles have not lost the power of responding to increases in venous pressures but, like the failing ventricles of a heart-lung preparation described by Starling (7), must operate under progressively higher venous pressures and progressively greater stretch in order to maintain their normal systolic discharge. Such a mode of failure certainly precipitated the irreversible circulatory failure in these dogs.

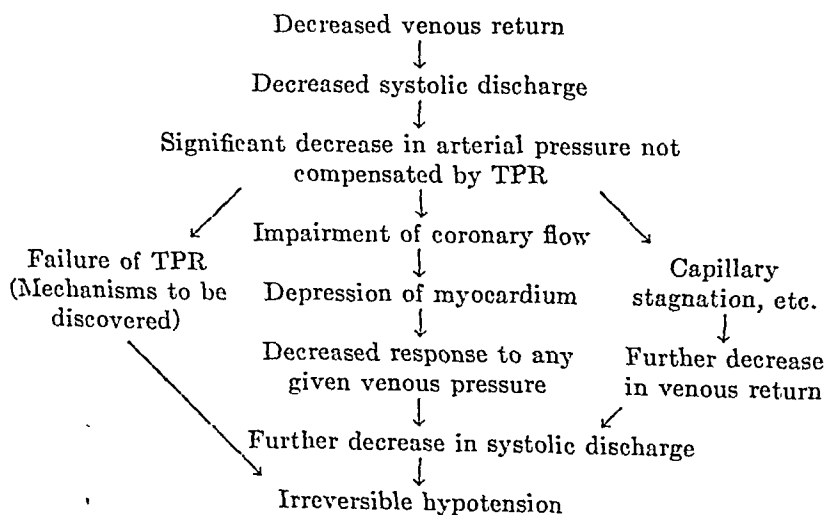
The similarities in the trends of venous pressure and arterial pressure pulses in dogs with closed and open chests suggest that such myocardial damage is at least not solely due to the more extensive operative procedures associated with the latter. Nor is it accounted for by the lowering of body temperature; many of our animals both with closed and open chests studied during the hot summer season died while temperatures were actually kept above normal. It is far more probable that the prolonged state of hypotension impairs the rate of coronary flow and while the work of the left ventricle is reduced, that of the right is probably not diminished significantly, with the result that failure of the right ventricle may precede that of the left. Admittedly, volume curves of the ventricles give no information concerning such a precedent failure of the right ventricle and our hypothesis for the present rests chiefly on observations of its dilated state through the glass cardiometer.

That such pronounced cardiac failure occurs in some dogs and not in others is readily explained when we realize that the initial condition of the myocardium

as well as the architecture of the vascular supply varies considerably in stray animals used. However, we cannot exclude the occurrence of myocardial depression even in those animals in which it is not a dominant sign. Its presence and the rôle it plays in determining irreversible failure may be concealed through concurrent reduction in venous pressures or default of the compensatory increase in TPR. When venous pressures are slightly reduced, we have no evidence as to whether the ventricles react as efficiently to the available stretching force as do the normal ventricles. The evidence from which we are accustomed to infer that the myocardium is not impaired in shock rests on repeated demonstrations that the heart is capable of responding to large infusions with increased output, but no one has demonstrated, as required according to Starling's law of the failing heart, that it responds as efficiently as a normal one to equivalent venous pressures.

In conclusion, two facts must be emphasized: 1. Our observations are limited to circulatory failure produced by a prolonged period of post-hemorrhagic hypotension and reinfusion of the withdrawn blood; they may not apply to other forms of shock. However, hemorrhage and infusion are bound to play a large rôle during warfare. 2. While our interpretations must be validated by other methods we feel justified in suggesting, on the basis of available evidence, that perhaps we have accepted too hastily the theory that the myocardium is unimpaired in shock and that the course of circulatory failure cannot be benefited by use of cardiac stimulants.

*A theory of shock.* Our conceptions are not in disagreement with the view that the sequence of events in shock begins with a decrease in effective circulating volume regardless of how this is accomplished. Decrease in venous return and mechanical decrease in systolic discharge as postulated by Y. Henderson and substantiated by Blalock and his associates remain early factors in initiating the sequence of events. However, unless compensatory mechanisms which increase TPR fail or unless the myocardium defaults, the precipitating mechanisms necessary to produce irreversible shock are lacking. The sequence of events which our experiments suggest may be diagrammatized somewhat as follows:





## SUMMARY

Dogs under morphine-barbital were bled until a marked state of hypotension was maintained for several hours. At the end of such a period the withdrawn blood (heparinized) was reinjected. A state of shock was considered to exist when such reinfusion failed to maintain arterial pressures for at least three hours and the upper intestines showed hemorrhagic changes at autopsy.

Cardiometer curves were recorded optically with aortic and venous pressures, and simultaneously on a kymograph as well. Changes in cardiac behavior were assessed from critically evaluated optical volume curves. Total peripheral resistance (TPR) was calculated from  $\frac{\text{mean pressure} \times 1332}{\text{cardiac output/sec.}}$  by use of calibrated drum records.

Experiments on 11 dogs consistently showed decreases in stroke and minute volumes during post-hemorrhagic hypotension and post-infusion failure, but the manner in which such reduction was occasioned differed. In one group, decrease in stroke was accompanied by decreasing venous pressures, in another it developed despite an elevation. Analyses of volume curves indicate that the capacity of the ventricles to respond to a given venous pressure (stretch) is reduced and that such hypodynamic action is masked when venous pressures decline concurrently. Prolonged reduction in coronary flow during severe hypotension is suggested as the cause. Our results strongly suggest that reduced capacity of the myocardium to respond to given venous pressures is one of the factors which precipitates an irreversible circulatory state.

In the majority of animals TPR increased during the period of post-hemorrhagic hypotension as well as after infusion, regardless of whether irreversible failure supervened or not. Such increase was never maximal, for stimulation of pressor nerves temporarily increased TPR tremendously. Dogs that showed little recovery of arterial pressure after initial hemorrhage, and some of those that developed rapid circulatory failure after reinfusion showed no increase in TPR. Consequently, our conclusion that development of hemorrhagic shock is not contingent on existence of high or low TPR. Persistence of an augmented TPR seems to retard rather than facilitate the development of hemorrhagic shock. Failure of a compensatory increase in TPR may be a second precipitating factor in creation of an irreversible state.

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# A STUDY BY QUANTITATIVE METHODS OF THE SPONTANEOUS VARIATIONS IN VOLUME OF THE FINGER TIP, TOE TIP, AND POSTERO-SUPERIOR PORTION OF THE PINNA OF RESTING NORMAL WHITE ADULTS<sup>1</sup>

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The circulation of the blood in small blood vessels has been regarded by pathologists, physiologists and biochemists as taking an important part in the processes of inflammation, temperature regulation and the distribution and collection of food and waste materials in varying amounts during rest and activity (1, 2, 3, 4). There is information which helps to explain some of the mechanisms through which the amount of blood flowing through a tissue can be adapted to its needs. But the impression prevails that except for part-time work in carrying out these functions, the blood vessels are just small inert tubes distributing blood. That this is far from the truth is becoming evident. The small vessels contain sympathetic nerve endings through the mediation of which arterioles and capillaries react presumably upon stimulation. The rôle of the venules is not yet understood. The tissues possess local and central mechanisms whereby the amount of blood in them is varied depending upon their presumed requirements. Such variations in volume of the vascular bed are of great importance to the general circulation. They have been noticed since the very first plethysmographic studies were made, but it is only recently that systematic attempts have been directed toward analyzing them. They have been shown to depend, in part, upon the temperature of the body (5), fluctuations in tone of the sympathetic nervous system and that of local vascular beds (6) as well as the degree of reactivity of the subject (7, 8). In addition it has been demonstrated that the size of an extremity, at least of the portion studied, decreases when individuals are stimulated by pain (9).

Repeated observations, each lasting an hour or longer, have now been made of the magnitude and rhythm of fluctuations of the tips of fingers and toes and of the postero-superior portion of the pinna. In this study the term "spontaneous variations in volume" refers to those changes in the peripheral vessels which arise from intrinsic adjustments rather than as a result of stimuli deliberately applied.

**MATERIALS AND METHOD.** Spontaneous variations in volume of fingers, toes and ears were recorded by the pneumoplethysmographic method of Turner (10) except that cellulose acetate, instead of metal, chambers were employed. This method is sensitive and can detect changes of 0.1 cu. mm. The

<sup>1</sup> This is the 1st paper reporting the results of studies of the small blood vessels and related subjects.

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chambers for the extremities were cylindrical (height approximately 3 cm.; radius approximately 1.2 cm.) (fig. 1 A) while that for the ear was hemicylindrical (height approximately 1.5 cm.), the radius being approximately 2.3 cm. An opening was cut to fit, without constriction, the shape of the pinna (fig. 1 B, C).

The temperature changes within the chambers, measured by constantan-copper thermocouples, were recorded simultaneously with the variations in volume. The volumes of the anatomical parts were measured by the method previously described (11). The size of the plethysmographic chambers was kept as small as possible. Although not directly utilizable in this study, changes in the effective volume of the chambers owing to the accumulation of perspired water were measured (12). Technically these measurements could not be made when anatomical variations were being studied but were under conditions as nearly comparable as possible.

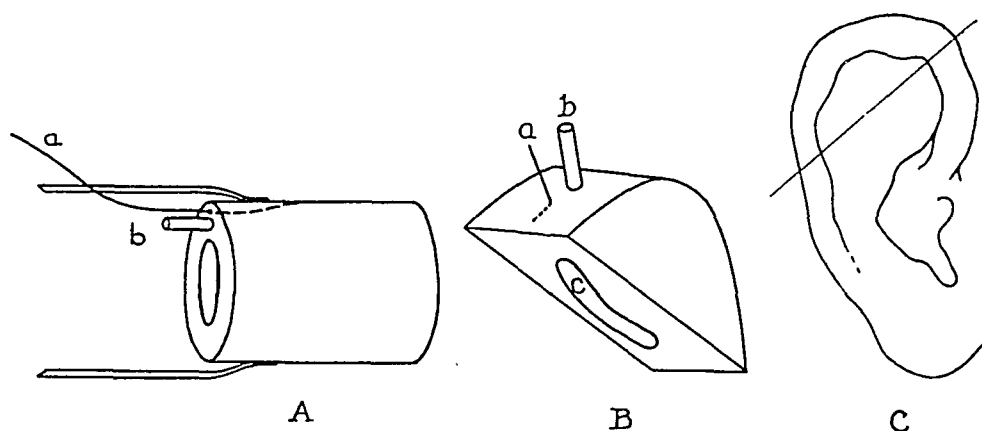


Fig. 1. Here are shown: A, the chamber for enclosing the tip of a finger or toe; B, the chamber for enclosing the postero-superior portion of the pinna (*a* represents the wires of the thermocouple; *b* is the tube leading to the recording capsule); C, the portion of the pinna lying above the oblique line which was studied.

The experiments were conducted in a medium sized air-conditioned laboratory (temperature  $75^{\circ}\text{F.} \pm 1$ ; relative humidity 50 per cent  $\pm 3$ ) which was semi-dark and sound proof. Sound within the room was limited to the almost inaudible continuous purr of air flowing from the air-conditioning unit. The room contained a hospital-type bed and the recording apparatus. It was otherwise unfurnished except for a few chairs, a large sink and a table.

Twelve normal white subjects (7 males, 5 females) varying in age from 22 to 44 years were examined. The records were made not earlier than two hours after meals. Three subjects were studied only once while the others were examined as often as two to fifteen times during the course of a year. Each subject, covered to suit his comfort, rested in bed for one hour before the chambers were placed on the tips of the right index finger and the right second toe and the postero-superior portion of the right pinna (fig. 1 C). The finger or toe tip is defined as that portion distal to a plane passing through the major distal dorsal and palmar or plantar skin creases. The parts were placed at the level

TABLE 1

*Measurements of the alpha and pulse waves in 12 normal white adults*

SUBJECT NO.	AGE	SEX	PART	VOLUME OF THE PART	VOLUME OF THE DEFLECTIONS OF THE ALPHA WAVES		FREQUENCY OF THE DEFLECTIONS OF THE ALPHA WAVES			VOLUME OF THE PULSE WAVES			
					Mean	Maximum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	
Group I 6	years	F	F* T* P*	cc. 3.5 4.2 2.0	cu. mm. per 5 cc. of part		no. per minute			cu. mm. per 5 cc. of part			
					2.4	7.1	8.8	11	7	1.6	2.0	0.9	
					2.1	7.1	6.3	10	2	1.0	1.2	0.7	
	33	32	M	F T P	5.0 2.7 2.7	4.7 4.0 3.7	18.0 12.8 14.7	9.2 7.0 10.0	12 9 11	5 5 7	2.8 5.7 1.1	3.3 6.6 1.5	2.5 5.0 0.9
	52	44	F	F T	3.2 3.0	7.1 3.7	23.3 15.1	5.8 6.8	9 10	4 6	9.8 4.2	12.4 5.2	7.5 3.0
	62	22	F	F T	3.1 3.6	9.3 5.0	29.0 12.4	12.2 9.8	14 10	10 9	5.2 3.7	5.8 4.0	3.7 3.4
	82	26	F	F T	4.2 3.0	4.8 5.1	12.0 26.4	8.4 7.6	10 9	7 7	3.4 2.8	4.5 3.3	2.4 2.0
Mean, maximum and minimum for group I			F T P		5.7 4.0 3.3	29.0 26.4 14.7	8.9 7.5 9.8	14 10 12	4 2 6	4.6 3.5 1.5	12.4 6.6 2.0	2.4 0.7 0.9	
Group II 67	40	M	F T P	6.2 4.7 2.5	12.4 8.1 7.3	32.0 25.4 19.3	7.0 9.2 4.5	8 13 8	5 5 2	5.5 3.7 2.2	7.7 4.3 2.5	3.6 3.2 1.9	
Group III 2	40	M	F T P	4.0 4.1 1.4	27.6 7.9 7.7	81.0 28.4 18.2	7.0 8.2 10.8	8 10 12	5 5 9	7.6 2.2 1.8	9.7 3.8 2.5	3.7 1.2 1.5	
3	32	M	F T P	4.5 4.5 2.3	20.9 8.7 7.0	53.0 24.5 24.0	6.6 7.6 12.4	9 10 13	5 6 10	9.4 3.8 9.9	11.0 4.5 10.5	5.5 3.1 9.2	
4	27	M	F T P	4.8 3.7 2.2	26.0 18.8 11.2	70.3 43.4 21.0	8.4 7.8 3.7	9 9 5	7 7 2	7.0 9.5 6.3	8.4 11.5 6.5	5.2 7.6 6.1	
17	23	F	F T	3.2 3.2	21.1 8.4	59.0 25.2	9.4 8.4	10 10	5 5	8.5 5.1	9.3 7.6	7.0 3.2	
38	31	M	F T P	4.6 4.3 1.9	22.1 10.7 6.5	62.5 34.5 18.0	7.4 7.8 9.0	11 10 11	5 5 6	5.5 4.6 5.4	6.8 5.5 5.9	3.9 4.3 5.1	

TABLE 1—*Concluded*

SUBJECT NO.	AGE	SEX	PART	VOLUME OF THE PART	VOLUME OF THE DEFLECTIONS OF THE ALPHA WAVES		FREQUENCY OF THE DEFLECTIONS OF THE ALPHA WAVES			VOLUME OF THE PULSE WAVES		
					Mean	Maxi- mum	Mean	Maxi- mum	Mini- mum	Mean	Maxi- mum	Mini- mum
					cu. mm. per 5 cc. of part		no. per minute			cu. mm. per 5 cc. of part		
65	39	M	F	4.1	15.7	47.0	4.5	6	2	3.9	4.1	2.9
			T	4.5	3.1	10.1	5.6	9	4	1.6	1.9	1.1
Mean, maximum and minimum for group III			F		22.7	81.0	7.2	11	2	7.0	11.0	2.9
			T		9.6	43.4	7.6	10	4	4.5	11.5	1.1
			P		8.1	24.0	9.0	13	2	6.1	10.5	1.5
Mean, maximum and minimum for all subjects			F		14.5	81.0	7.9	14	2	6.9	12.4	0.9
			T		7.1	43.4	7.7	13	2	4.0	11.5	0.7
			P		6.6	21.0	8.6	13	2	4.1	10.5	0.9

\*F = right index finger tip.

T = right second toe tip.

P = postero-superior portion of right pinna.

of the subject's heart. At first each study lasted three hours, but when it was observed that for the current purpose little was gained by such prolonged observations, the period was reduced to one hour.

The values obtained have been expressed in cubic millimeters per five cubic centimeters of the anatomical part studied.<sup>3</sup> Five cubic centimeters was chosen because it approximates the average volumes of the parts to be studied (11, 13). All observed values have been calculated so as to be comparable; changes are therefore given as if 5 cu. mm. were the size of fingers, toes and pinnae of persons of various dimensions (table 1). Corrections were made for temperature changes within the chamber.

RESULTS. Fingers, toes and pinnae were found to undergo continuous variations in volume. The spontaneous changes which occurred ranged from less than 0.1 cu. mm. to 350 cu. mm. or more. These fluctuations fall naturally into 5 easily recognizable rhythms (figs. 2, 3).

1. *Pulse waves.* These were of course occasioned by the heart beat. Their volume varied markedly (table 1, fig. 4). It was noticed that there was a relationship<sup>4</sup> between the size of the pulse waves and that of the alpha waves described later. Measurements were made at 4 successive 5-minute intervals. The mean volumes were 6.9 cu. mm. in the finger tips, 4.0 cu. mm. in the toe tips, and 4.1 cu. mm. in the pinnae. The pulse waves in the finger tips were accordingly twice the size of those in the toes and the postero-superior portion of the pinnae.

2. *Respiratory waves.* The volume of the vascular beds of the three parts

<sup>3</sup> In this paper, changes in a part are given as cubic millimeters per 5 cc. of part.

<sup>4</sup> This subject will be presented in detail in another publication.

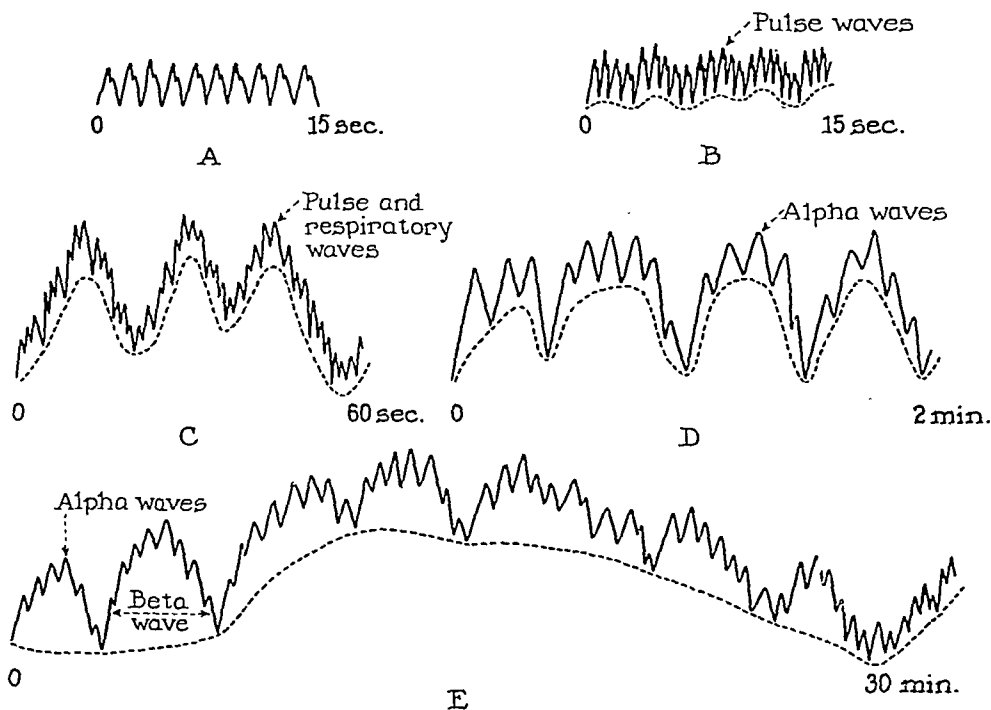


Fig. 2. Schematic drawings of 5 types of spontaneous volume waves are shown: A. *Pulse waves* are the volume changes occasioned by the heart beat. B. *Pulse waves* and *respiratory waves*. The latter are indicated by the interrupted line. C. *Pulse waves*, *respiratory waves* and *alpha waves*. The latter are indicated by the interrupted line. D. *Alpha waves* and *beta waves*. The pulse and respiratory waves are not shown. The *beta waves* are indicated by the interrupted line. E. *Alpha*, *beta* and *gamma waves*. The pulse and respiratory waves are not shown. The *gamma waves* are indicated by the interrupted line.

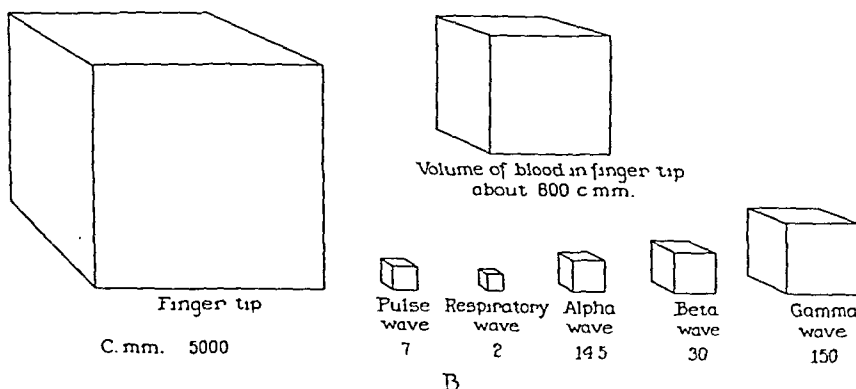
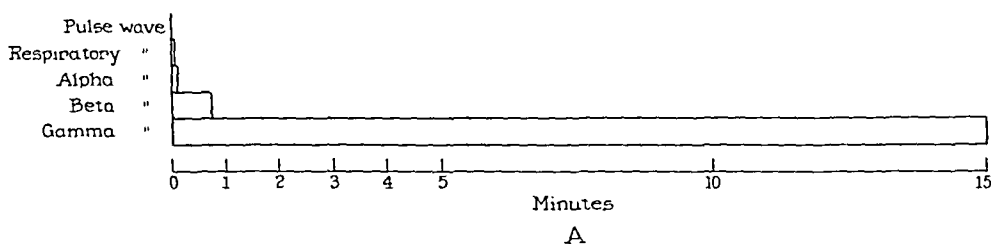


Fig. 3. A, the relative time values of 5 waves are represented by a histogram; and B, their average relative volumes by cubes of corresponding dimensions.

varied with the normal respiratory cycle. This was noticed most frequently in the pinnae but rarely in the toes. The waves occurred either in one part, in two, or in all three simultaneously (fig. 5 a). They varied from subject to subject and also from time to time in each subject, the values ranging from 0.1 to 5 cu. mm.

A special type of variation in the fingers and toes occurred within a few seconds after a deep inspiration. This form has been studied by many investigators (5, 6, 7, 14). It may exhibit several characteristics: 1. The deep inspirations responsible for the change occurred spontaneously and not as consequence of instruction or after stimulation. Shortly after them there was a sudden decrease in volume (fig. 5 B). This varied from 5 to 105 cu. mm. Associated with this the pulse wave decreased in volume. After a few pulse beats vasodilatation

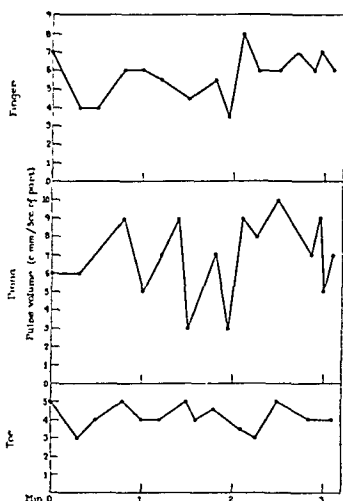


Fig. 4. These three curves show variations in volume of the pulse waves recorded simultaneously in the three anatomical parts. There are marked variations in volume of the waves in each part and discordant variations among the parts.

began and the volume gradually increased to its earlier dimension. Small variations (2 to 8 cu. mm.) similar to the alpha waves described later made their appearance just before vasodilatation was complete. Alpha waves conforming to the pattern characteristic of the subject then became reestablished. 2. In general, the variations which depended upon deep inspiration were more prominent in the fingers than in the toes. 3. After two or more successive deep inspirations, their effect diminished until no additional response was noticed; the shorter the interval the less did the volume change. As before, the initial pattern returned. 4. The change in the pinnae was not as definite, predictable, nor as large as in the fingers and toes, nor was it necessarily concordant. There was either an increase or a decrease followed by a change in the opposite direction. These changes were similar to those described by Hertzman and Dillon (6). In one individual, a rare exception, a deep inspiration did not always produce these changes (fig. 5 c).

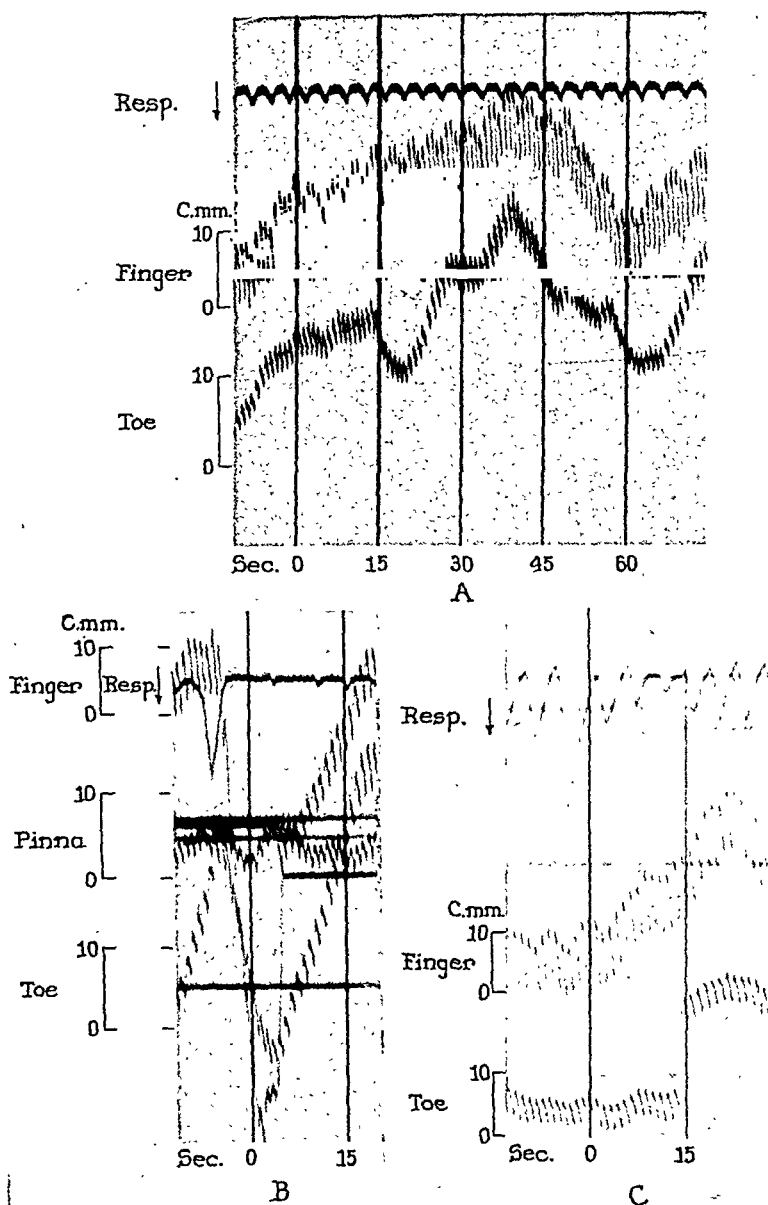


Fig. 5. The influence of respiration on volume curves is shown: A. Normal changes in the volume of the finger related to respiration. B. Sudden decrease in volume of the tips of the fingers and toes and of the pinna immediately following deep inspiration. C. Absence of vasoconstriction following deep inspiration. A downward movement of the respiratory tracings indicates inspiration. The changes are cubic millimeters per 5 cc. of part.

3. *Alpha waves*.<sup>5</sup> Less frequent than respiratory waves were variations to be called alpha waves. They occurred in all parts studied in all subjects. In

<sup>5</sup> The fact should be emphasized that as physiological realities waves do not exist, they are in this connection plethysmographic representations of complex occurrences. Upward deflection corresponds to increase in volume, downward, to decrease.



the absence of sudden changes in volume their ascending and descending limbs were of smooth contour and about equal in size; it was not at all uncommon to encounter series of unequal size. Large increases in the size of a part commonly took place slowly and usually in steps, whereas large decreases usually occurred in one long rapid deflection. In order to obtain quantitative information about

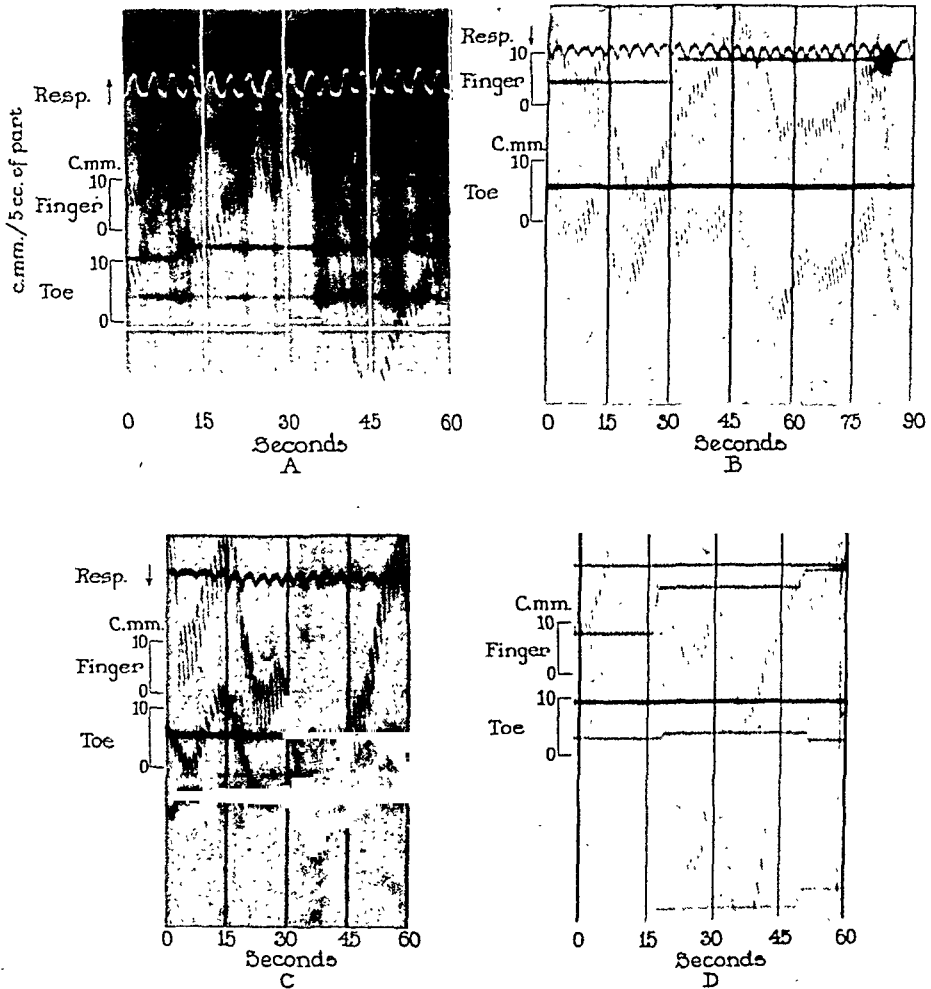


Fig. 6. Records of an individual, taken at long intervals, are closely similar, as shown in tracings of the tips of a finger and a toe at various times: A. December, 1939. B. February, 1940. C. ninety minutes after B. D. January, 1941. The volume changes are cubic millimeters per 5 cc. of part.

alpha waves, it was more practical to measure the size and frequency of their limbs rather than of the whole waves (fig. 6 A). The mean frequency of the total number of deflections (including both upward and downward) was 7.9 per minute in the fingers, 7.7 per minute in the toes, and 8.6 per minute in the pinnae. It is striking that the number observed in the three parts was not identical. The mean volume of the total number of deflections was 14.5 cu. mm. in the fingers, 7.1 cu. mm. in the toes, and 6.6 cu. mm. in the pinnae (table

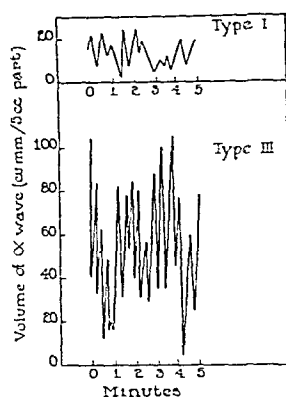


Fig. 7. Diagrams are shown to illustrate the difference between type I and type III alpha waves.

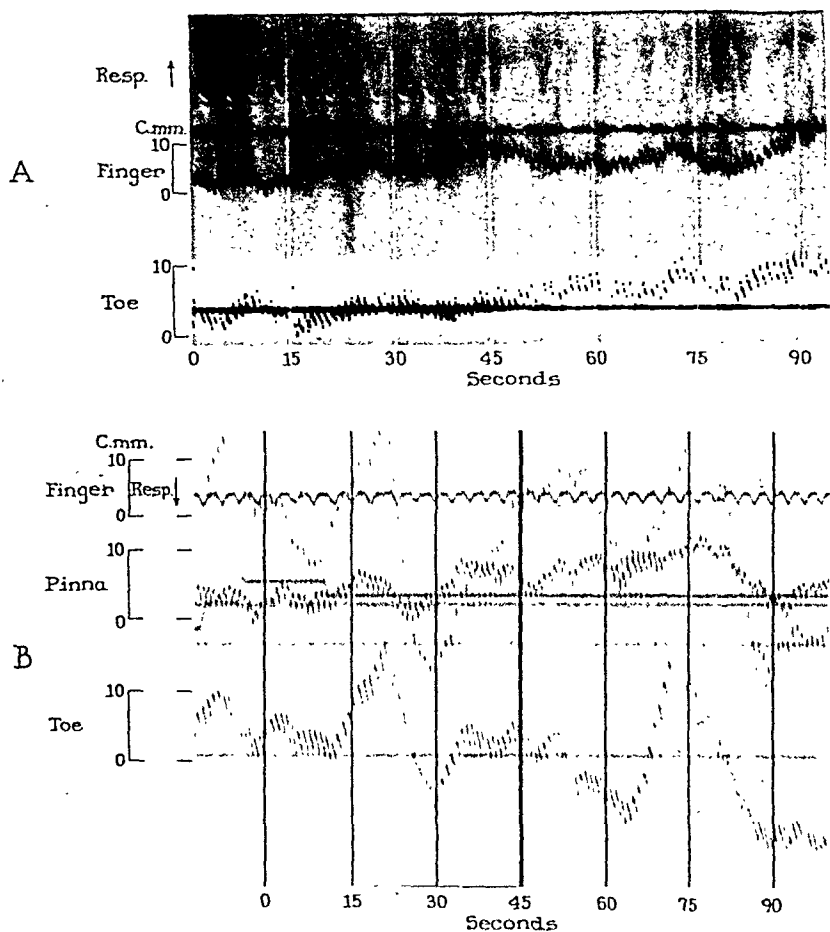


Fig. 8. Records are reproduced to illustrate typical differences: A, original tracings from a patient with type I alpha waves and B, from a patient with type III alpha waves. Inspiration is indicated by the direction of the arrows. The changes in volume are cubic millimeters per 5 cc. of part.

1). The minimum value of a single alpha deflection depends, of course, upon the sensitivity of the recording instrument. All values less than 0.5 cu. mm. were included arbitrarily in the 0.5 cu. mm. category.<sup>6</sup> The maximum deflection recorded was 81 cu. mm. The frequency varied from two to fourteen per minute. No correlation between frequency and volume was noticed.

The variations in the size of the limbs of alpha waves were typical of groups of

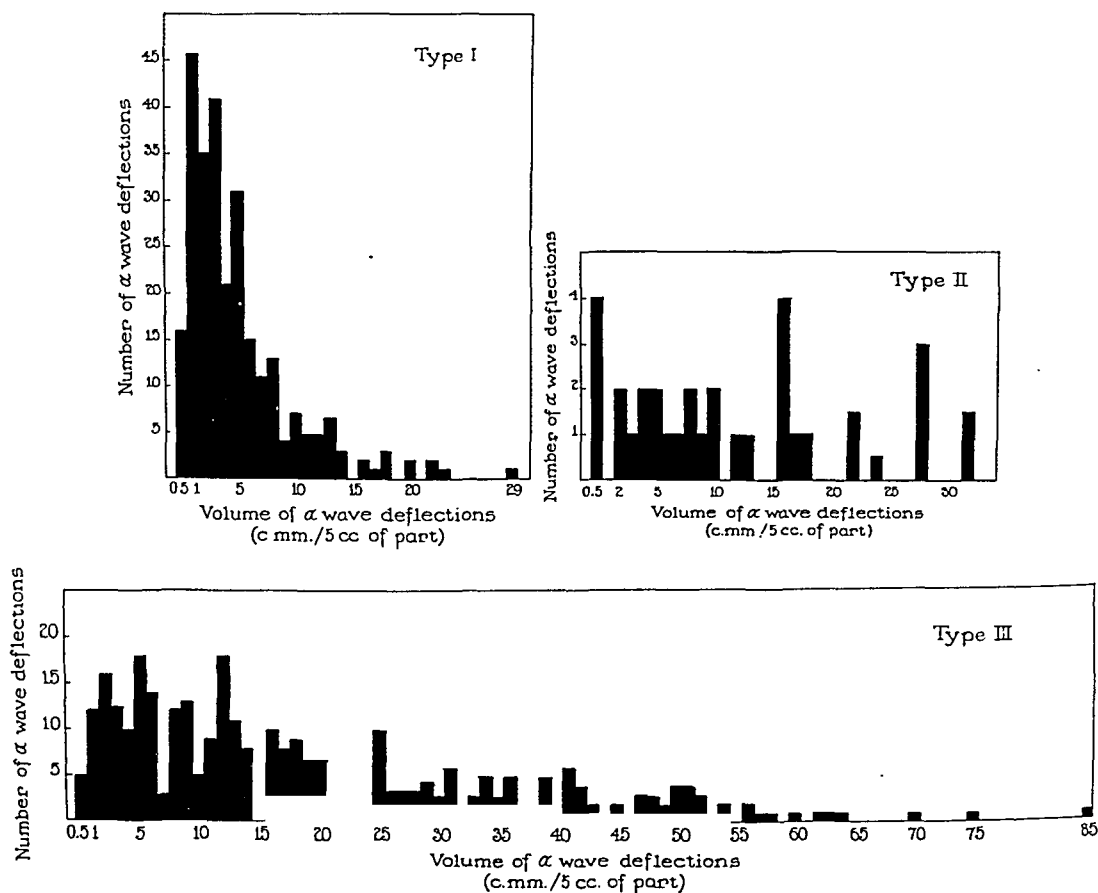


Fig. 9. Three charts are shown to demonstrate the difference in the distribution in size of alpha waves in different groups of persons: *a*, in individuals of type I, most of the alpha waves in the finger tip during a 15 minute period in 5 subjects are small; *b*, in type II, in one subject, the sizes on the whole are larger but more scattered; *c*, in type III, in six subjects, they are also larger and much more scattered.

persons, without being characteristic of individuals (fig. 6). The curves obtained from the finger tips of twelve subjects fell into three easily identifiable types. In type I (5 subjects) almost all of the deflections represented less than 6 cu. mm. and all less than 29 cu. mm. (figs. 7, 8 and 9 A). Their sizes varied within relatively narrow limits with a mean of 5.7 cu. mm.

Characteristic of type III (6 subjects) on the other hand (fig. 9 C) was the wide range in size of the deflections, many being greater than 30 cu. mm. and a

<sup>6</sup> In these calculations, volumes less than 0.5 cu. mm. have no significance.

few greater than 45 cu. mm. The mean was 22.7 cu. mm. The waves of one subject, type II, were intermediate between those of type I and type III (fig. 9 B.)

The alpha waves of the toes and pinnae can in a general way be divided into three types, but with less satisfactory results than in the case of the finger tips. The alpha waves were concordant variations in all three anatomical parts for three-quarters of the time. In the other quarter the record from one or two parts showed opposite, extra, or no changes (fig. 8, table 1). Even though the changes in all the parts were unidirectional most of the time, the amount tended to be very different.

4. *Beta waves.* The succession of alpha waves was superimposed upon larger

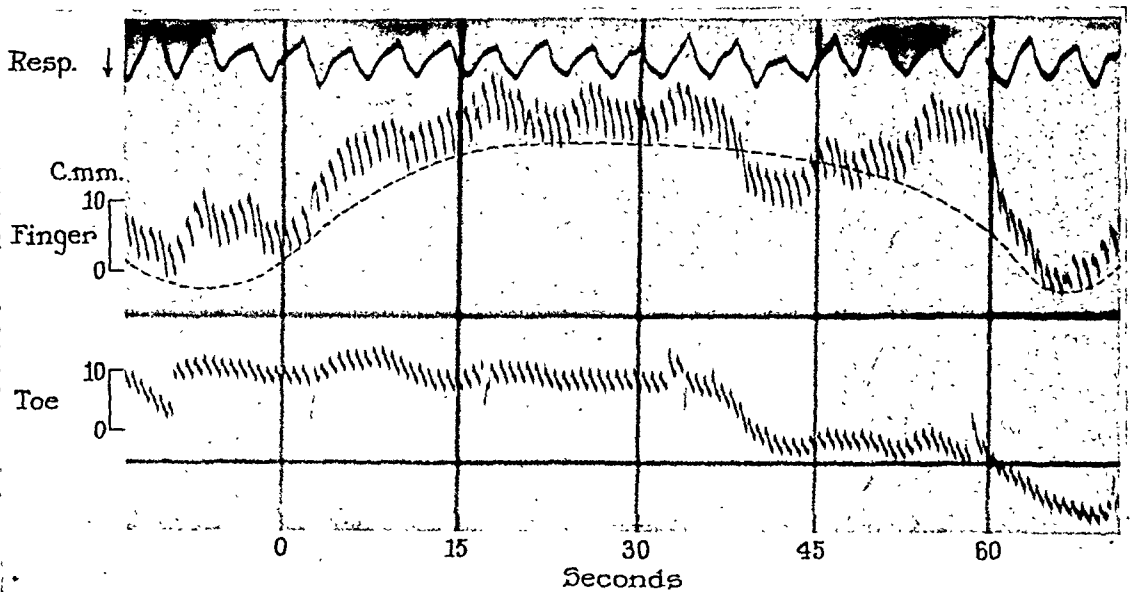


Fig. 10. A beta wave in the finger tip is indicated by the interrupted line. The duration of this beta wave is approximately 60 seconds.

waves to be called beta waves (fig. 10). The frequency of deflections was 1 to 2 per minute and the size, 5 to 60 cu. mm. All subjects and all parts studied exhibited beta waves, but there was no clearly defined general pattern as in the case of alpha waves. Their rhythm was totally irregular, but simultaneous deflections of fingers and toes and of pinnae tended to vary in the same direction.

5. *Gamma waves.* These represented slowly developing, but extensive changes in volume which were in effect the base line for the beta waves (fig. 11). The number of deflections varied between one and eight per hour and the size between 50 and 350 cu. mm. Naturally only a very small number of gamma waves were recorded in the course of the observations of any one patient, but it was evident that simultaneous gamma deflections in finger, toe and ear tended to be unidirectional.

None of the five waves described can be considered representative of an independent volume change. Each of them beginning with the pulse wave and

continuing in order through the respiratory, alpha, beta and gamma waves, is imposed in turn upon the waves next larger in size. Rhythms, the waves of which may be of greater excursion and of longer duration, may exist though they have not been detected in these studies (fig. 11).<sup>7</sup>

In the period during which a record was obtained, the temperature within the plethysmographic cups varied less than 1 degree and at all times changed relatively slowly. The maximum temperature change could have altered the volume of 10 cc. of air, the largest dead space in any experiment, by not more than 36 cu. mm.

During the course of each experiment there was an accumulation of perspired water. The methods used in this study precluded the possibility of estimating simultaneously the elimination of water. But the amount can be calculated, from data previously reported (12). During an hour's observation approximately 76 cu. mm. of water are secreted by the finger tip, 41 cu. mm. by the toe

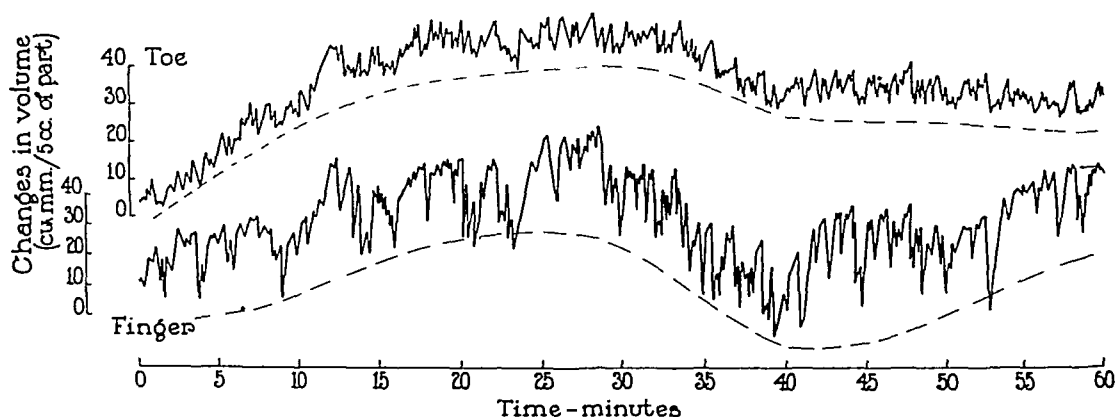


Fig. 11. The gamma waves in the tips of a finger and toe are indicated by the interrupted line. The duration of this gamma wave is 40 minutes.

tip and 25 cu. mm. by the postero-superior portion of the pinna. It appears impossible that either temperature changes or accumulations of water could modify significantly the size of any of the waves, though in an observation extending over hours the base would be slowly elevated.

**DISCUSSION.** In a quantitative study of spontaneous variations in volume of certain anatomical parts it was found possible to identify and to measure the waves to which the fluctuations in volume gave rise. Arbitrary terms, alpha, beta and gamma were attached to three of the waves until a more descriptive terminology becomes available based on an understanding of their mechanism. At times beta waves were difficult to identify and seemed to represent variations in alpha waves rather than independent waves with alpha waves superimposed

<sup>7</sup> Of the authenticity of rapidly recurring waves there is no question. There is a question whether the waves of slow rhythms may not be artefacts owing to movements of the cups. The technique employed renders this possibility unlikely. The concordance of occurrence of waves taken from the three parts is itself evidence that physiological manifestations are being recorded.

upon them. This was possible whenever alpha waves took on dimensions approximating beta waves. In spite of such instances the beta waves were definite nevertheless, and were present in all subjects at every observation.

Although relatively prolonged, these observations were not conducted for periods of time sufficiently extended to give enough information about the gamma wave. If after three or more hours the subjects became fatigued, fatigue itself would have modified the volume changes. But in a relaxed subject five minutes of continuous recording furnished data necessary for the characterization of *alpha* waves and fifteen minutes for *beta* waves.

Although the subjects were well acquainted with the apparatus and the method used the effect of the period of rest before affixing the cups to fingers, toes and ears, was repeatedly nullified by the manipulation of applying them. Delay of another period of approximately thirty minutes was necessary before most subjects could be considered sufficiently relaxed. During this half-hour, records were made nevertheless, but the pulse waves were small and the temperatures in the chambers surrounding the parts relatively low, both events indicative of vasoconstriction. Not until vasoconstriction disappeared did the alpha, beta and gamma waves attain their full development. The measurements now presented represent periods when subjects were comfortable and relaxed. The continuous low purr of the air-conditioning unit and the comfortable atmospheric conditions contributed toward progressive relaxation. Naturally at no time was it possible to control or to be aware of what was going on in a subject's mind, but it was observed that his occasional spontaneous remarks were reflected shortly afterward by marked decreases in volume, particularly if an unpleasant thought crossed his mind such as the possibility that the apparatus might reveal the presence of a disease or that prolongation of the study would make him late for an appointment. To test this possibility the observers would ask relevant questions toward the end of each recording. Almost without fail, an unpleasant subject of discussion resulted in rapid and large decreases in volume.

In studies such as this of the external parts of the body the apparatus records the sum of a number of changes. These are related to a number of factors: 1, variations in volume of the vascular bed itself; 2, variations in volume of the air enclosed in the plethysmographic chamber owing to temperature changes; 3, accumulation of perspired water in the enclosing chamber, and 4, variations in volume of the inter- and intra-cellular fluids.

Corrections have been made for changes in temperature of the air immediately surrounding the parts. The variations in volume of the inter- and intra-cellular fluids and the relative importance of arterioles, capillaries, venules and arterio-venous communications were not ascertained. It is difficult to conceive how changes in the volume of the extravascular fluids could take place with the speed and to the extent of those recorded for each anatomical part.

The volume changes represented by the ascending and descending portions of the alpha waves fell into three types. In one (type I) they were small and the variations relatively limited. In another (type III) they were large and of

greatly variable magnitude. In one (type II) they were moderate in size, intermediate between type I and type III. The individuals with type I were placid and emotionally stable people. They were observed to adjust themselves easily to the new situation of being subjected to a physiological procedure. Some were associates in the laboratory so that it was possible to observe their reactions to the numerous and sometimes upsetting events developing in the course of a day. Subjects exhibiting type I could be depended upon to make little of disturbing environmental conditions. Those with type III reacted to their environment with wide fluctuations in mood. They were inquisitive and at times anxious about the experimental procedure. It was necessary to ask them numerous times to remain quiet, not only the first but on subsequent occasions long after they should have become accustomed to the procedure. After repeated observations no instance was found when an individual with type I waves exhibited type III waves, or vice versa.

The existence of this correlation between disposition and variations in the type of alpha waves is not surprising. Marked vasomotor changes, such as constriction or dilatation of certain blood vessels, increase or decrease in blood pressure and attacks of cardiac pain or coronary occlusion are known to be frequently precipitated by emotional strains such as fear, joy or worry. Marked changes in the volume of peripheral blood vessels are known likewise to follow pain, heat, cold, hunger and thinking. The observations now described tend to show that fundamental differences exist in the reactivity of blood vessels in types of people even when no external strain is being imposed.

The fact that there are simultaneous increases and decreases in widely separated superficial portions of the body (finger tip, toe tip and postero-superior portion of the pinna) while the circulating blood volume supposedly remains relatively constant suggests the probability of a shift back and forth of blood between superficial and internal parts of the body. Studies by others (15, 16, 17, 18, 19) have shown that in dogs there are spontaneous variations of splenic volume of a magnitude and frequency comparable to that of beta and gamma waves. The changes in the spleen as well as in other viscera may be reciprocally related to changes in the superficial portions of the body. "Spontaneous" fluctuations documented by the alpha, beta and gamma waves possibly aid in the distribution and mixing of all of the extravascular fluids and may facilitate mechanically the passage of fluid through capillary and lymphatic walls.

The mechanisms related to the alpha, beta and gamma waves are, as has been said, unknown. They are almost necessarily related to functions of the autonomic and central nervous systems and also, probably passively, to the blood pressure (5, 6, 7, 8). No data are available which associate these waves with these functions.

#### SUMMARY

The spontaneous variations in volume of the tip of the right index finger, the tip of the right second toe and the postero-superior portion of the right pinna of 12 normal white adults have been studied quantitatively. All parts

have undergone continuous variations in blood volume which consist of at least five separate rhythms. The effects of the heart beat and respiration were reflected in the pulse waves and respiratory waves respectively. The three other waves were arbitrarily named alpha, beta, and gamma.

The mean frequency of the alpha waves was 7.9 per minute in the finger tip, 7.7 in the toe tip, and 8.6 in the pinna. The mean volume of the deflections was 14.5 cu. mm. per 5 cc. of finger, 7.1 cu. mm. per 5 cc. of toe and 6.6 cu. mm. per 5 cc. of pinna. The frequency of the beta deflections varied from one to two per minute and the size from 5 to 60 cu. mm. per 5 cc. of tissue. The number of gamma deflections varied from one to eight per hour and the volume from 50 to 350 cu. mm. per 5 cc. of tissue.

The alpha waves obtained from the finger tips of the 12 subjects fell into three types. In type I (5 subjects) the deflections were relatively small and varied very little in size. Type III (6 subjects), on the other hand, showed a wide spread in the sizes of the deflections, many being large. Type II waves, found in a single subject, were intermediate between those of type I and type III. The subjects with type I were phlegmatic and stable while those with type III were excitable and exhibited wide fluctuations in mood.

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# A STUDY OF THE RELATIONSHIP BETWEEN THE PULSE AND ALPHA WAVES OF THE TIPS OF THE FINGERS AND TOES OF FIVE ADULTS<sup>1</sup>

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Burton (1) has pointed out that spontaneous variations in the volume of small peripheral parts, such as fingers and toes, dependent upon the blood flow, were very large and consistently in the same direction. He stressed his belief that their essential rôle was one of temperature regulation. Hertzman and Dillon (2) have also studied such variations.

In a previous communication (3) five different kinds of waves were described documenting spontaneous variations as obtained by a plethysmographic method. Excluding the pulse and respiratory waves, the most frequently occurring ones; named alpha waves, have deflections which commonly are unidirectional in the fingers and toes and postero-superior portion of the pinna, but a more exact analysis was not made.

For more detailed study the records of five resting male subjects (4 normal, 1 senile) were selected because the usual concordant relationship, whether increase or decrease, of pulse wave to alpha wave was not uniformly observed. At the same time changes in size of alpha deflections were compared with those of pulse waves in the fingers and toes. The 5 minute period showing the most marked discrepancy was chosen for this study. Only the direction of variation in volume was used as the criterion of concordant variations in each of the four categories (fig. 1).

Simultaneous *alpha deflections and pulse waves* in the finger tips<sup>3</sup> were concordant in 34 per cent, and in the toe tips in 71 per cent. The range was 22 to 50 per cent in the finger tips and 62 to 76 per cent in the toe tips. In the finger and toe tips simultaneous *alpha* waves were concordant in 56 per cent, the range being 50 to 62. The *pulse* waves were concordant in 45 per cent. Forty-five per cent of the simultaneous variations in the size of the pulse waves in the finger and toe tips were concordant.

Concordant variations in size of alpha deflections and pulse waves were startlingly low in the finger tips, while the percent in the toe tips agreed in general with the previous estimates. It is not possible to assign a reason for this apparent discrepancy. In other portions of the same records, in other records of the same subjects, and in the records of other subjects it was not present. The

<sup>1</sup> This is the 2nd paper reporting the results of studies of the small blood vessels and related subjects.

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<sup>3</sup> It was always the tips of the right index finger and of the right second toe that were examined.

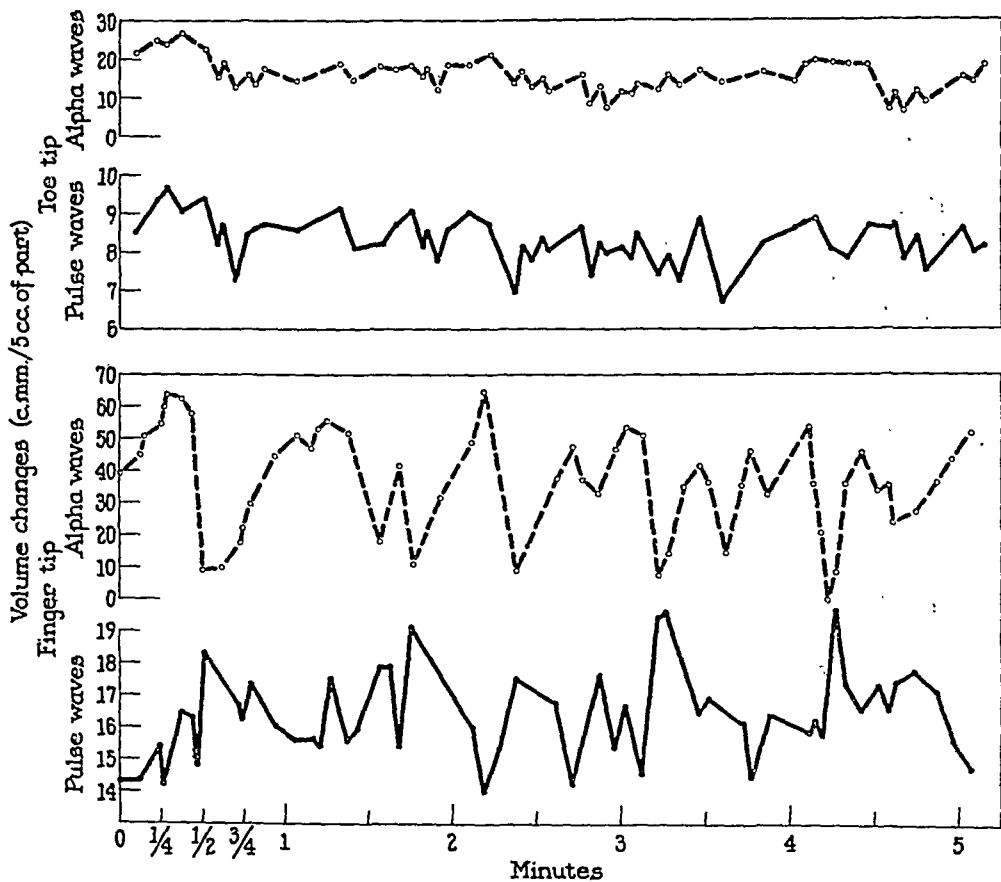


Fig. 1. Relationships are shown between the volumes of the alpha waves and pulse waves of the tips of the fingers and toes recorded simultaneously in one subject. There is marked discordance in the direction of the variations in volume of any combination of the waves of the two parts.

TABLE 1

*The per cent of concordant variations in volume of the alpha and pulse waves, the alpha waves, and the pulse waves in the finger and toe tips of 5 adult male subjects*

SUBJECT NO.	AGE	ALPHA WAVE		ALPHA WAVES OF FINGER AND TOE TIP	PULSE WAVES OF FINGER AND TOE TIP	DIAGNOSIS
		To pulse wave of finger tip	To pulse wave of toe tip			
	years	per cent	per cent	per cent	per cent	
2	40	36	69	62	39	Normal
3	32	22	76	50	52	Normal
35	83	33	62	59	48	Senile
38	31	50	76	52	41	Normal
90	35	31				Normal
Mean		34	71	56	45	

per cent of concordant variations of the alpha deflections and of changes in size of pulse waves of finger and toe tips was so close to 50 as to suggest no more than a chance relationship. In fact when the variations in alpha waves of the

finger of one subject were compared with those of another, the percentage of concordant changes was like that in the fingers and toes of the same subject. *Pulse* waves of two subjects told the same story. No improvement in correlation could be achieved by comparing other waves in the records of those selected. Whether this attempt should be made because an influence on the waves may occur at different times in the fingers and toes is open to doubt. Usually, in the resting, uninfluenced subject, physiological influences occur simultaneously in both. Even when the changes in any of the four categories were unidirectional, the amount was rarely the same, suggesting that the underlying mechanism, whether central or peripheral, is capable of manifesting independent activity in widely separated anatomical parts. In addition, great changes in the size of a part, as those occasioned by alpha waves, may occur with only slight and temporary changes in the size of the pulse waves.

*Alpha* deflections were of the usual order of magnitude and average frequency (3). Especially prominent were the marked differences in the duration of successive alpha waves as well as of those starting at nearly the same time in fingers and toes. At no time did the size of a part remain constant for more than a few pulse beats.

During a brief period of time, the *basic* size of a part may remain unchanged or it may change. If it changes, it must do so either by the accumulation or by the loss of fluid, change which can conceivably be documented by the size of alpha (and other) waves during a preceding interval. The size of a part is influenced, furthermore, during any period, by fluctuation in the volume of fluid imported into that part, again as shown by the size of the waves, especially alpha waves. A metaphor would be the influence of waves of the same size on the total configuration at high and at low tide (fig. 1). But more important than the variability of any one of the waves is the apparent lack of correlation between any two varieties as for example pulse or alpha waves. Each type of change appears to be independent. What this means in the body's economy remains obscure, but the fact that such variability can occur, even if it may be less prominent at times, would argue that local control over these waves may play a significant rôle and that vessels other than small arteries and arterioles probably exert an influence upon the size and direction of change documented by the alpha waves.

These mechanical arrangements are a background for the situations which permit appropriate accommodations in metabolism. There are involved increases and decreases in the transport of fluid in and out of anatomical parts, but also the residence of fluids in tissue spaces, in lymphatics and in other preformed locations.

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# A STUDY BY QUANTITATIVE METHODS OF THE SPONTANEOUS VARIATIONS IN VOLUME OF THE TIPS OF THE FINGERS AND TOES AND POSTERO-SUPERIOR PORTION OF THE PINNA OF HYPERTENSIVE PATIENTS AND SENILE SUBJECTS<sup>1</sup>

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Spontaneous variations in volume of the tips of the index finger and of the second toe and of the postero-superior portion of the pinna of normal resting subjects, as studied by the pneumoplethysmographic method of Turner (1) have been classified in five separate rhythms (2). The heart beat and respirations were shown to be documented in these "spontaneous" variations. The waves constituting three other rhythms could not be related to easily recognizable influences and were arbitrarily named alpha, beta and gamma.

In the present investigation a group of patients with arterial hypertension and a group of senile subjects were studied to see if there were a relation between the spontaneous variations and the nature of their ailments. The patients included 7 females with "diencephalic" hypertension varying in age from 29 to 54 years, 2 males and 4 females with "renal" hypertension varying in age from 27 to 46 years, and 7 senile males with general arteriosclerosis varying in age from 70 to 90 years.

The clinical criteria of "diencephalic" hypertension as described by Page (3) were adopted in selecting patients of this group. The essential signs are blotchy flushing of the face, neck, chest, and at times of the abdomen, watering of the eyes, excessive perspiration, palpitation and tachycardia, increase in blood pressure and coldness of the extremities. The patients with arterial hypertension of renal origin were suffering apparently as the result of chronic hemorrhagic Bright's disease or chronic pyelonephritis.

These patients were under treatment in this Hospital for periods ranging from several months to years. None of the hypertensive patients was suffering from cardiac failure or more than slight impairment of renal function.

The peripheral arteries of the senile subjects were thickened and tortuous and there was arteriosclerosis of the retinal vessels. There was no diastolic hypertension. The systolic blood pressure was 165 mm. of mercury or less. They were free from congestive cardiac and renal failure. They were up and about maintaining an active daily schedule.

The method and apparatus employed in this study were the same as those used in investigating normal individuals (2).

**RESULTS.** The waves previously described as "spontaneous" variations

<sup>1</sup> This is the 8th paper reporting the results of studies of the small blood vessels and related subjects.

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in volume of peripheral parts of normal subjects were demonstrable in all of the patients. These included the pulse, respiratory, alpha, beta, and gamma waves.

TABLE 1

*Measurements of the pulse and alpha waves in patients with diencephalic hypertension*

SUBJECT NO.	AGE	SEX	BLOOD PRESSURE	PART	VOLUME OF PART STUDIED	VOLUME OF THE DEFLECTIONS OF THE ALPHA WAVES		FREQUENCY OF THE DEFLECTIONS OF THE ALPHA WAVES			VOLUME OF THE PULSE WAVES		
						Mean	Maximum	Mean	Maximum	Minimum	Mean	Maximum	Minimum
	years		mm. Hg		cc.	cu.mm./5 cc. of part		No. per minute			cu. mm./5 cc. of part		
20	30	F	230/134	F* T*	3.6	13.5	37.9	8.8	11	6	7.9	10.7	5.4
					4.3	9.2	21.0	8.4	12	7	4.8	7.1	3.1
59	41	F	200/120	F	5.3	9.5	24.6	8.8	10	8	3.5	4.3	2.8
				T	3.4	5.4	14.6	7.6	9	6	1.5	1.8	1.2
				P*	2.2	2.8	6.9	14.6	15	10	5.6	6.2	4.6
70	54	F	230/120	F	3.9	10.7	34.6	7.8	9	7	5.9	7.2	4.6
				T	3.0	5.3	14.1	9.6	11	7	4.3	4.5	3.6
75	29	F	210/122	F	4.0	13.5	35.0	9.0	10	8	5.6	7.0	3.9
				T	3.9	8.4	17.8	8.0	10	7	3.7	4.9	2.2
				P	1.9	5.9	18.9	10.6	12	9	2.7	3.2	2.1
81	38	F	220/130	F	3.1	22.5	65.7	7.8	11	6	8.0	8.9	6.6
				T	3.4	15.7	67.6	7.8	10	6	5.0	9.3	1.5
				P	1.2	6.0	12.2	11.8	16	9	3.4	3.4	3.0
85	40	F	192/120	F	4.2	8.4	42.1	11.0	13	10	7.3	8.2	6.0
				T	4.0	7.1	31.3	7.6	11	5	1.6	2.2	1.3
				P	1.4	3.1	6.9	11.6	14	10	1.4	1.4	1.0
86	33	F	218/142	F	3.8	15.2	60.5	7.6	11	6	6.8	7.5	6.4
				T	3.9	7.6	36.9	8.8	11	6	2.3	3.4	1.5
				P	1.9	5.6	10.6	8.6	10	6	1.6	2.1	1.3
Mean, maximum and minimum for the group				F		13.3	65.7	8.7	13	6	6.4	10.7	2.8
				T		8.4	67.6	8.3	12	5	3.3	9.3	3.6
				P		4.7	6.9	11.4	16	6	2.8	6.2	1.0

\* F = Right index finger tip.

T = Right second toe tip.

P = Postero-superior portion of the right pinna.

*Pulse waves.* In *diencephalic hypertension* the mean volume of the pulse wave was 6.4 cu. mm. per 5 cc. of finger tip,<sup>3</sup> 3.3 cu. mm. in the toe tip and 2.8 cu. mm. in the pinna (table 1). In *renal hypertension* it was 8.2 cu. mm. in the fingertip, 4.2 cu. mm. in the toe tip, and 6.5 cu. mm. in the pinna (table 2); and in

<sup>3</sup> All measurements of volume are in cubic millimeters per 5 cc. of part.

senility it was 6.0 cu. mm. in the finger tip, 2.0 cu. mm. in the toe tip and 4.6 cu. mm. in the pinna (table 3).

*Respiratory waves.* These waves were observed during quiet breathing in the three parts (fingers, toes and ears) of most of the patients. They were essentially the same as in normal individuals. There were no differences among the three groups of patients.

TABLE 2

*Measurements of the pulse and alpha waves in patients with renal hypertension*

SUBJECT NO.	AGE	SEX	BLOOD PRESSURE	PART	VOLUME OF PART STUDIED	VOLUME OF THE DEFLECTIONS OF THE ALPHA WAVES		FREQUENCY OF THE DEFLECTIONS OF THE ALPHA WAVES			VOLUME OF THE PULSE WAVES		
						Mean	Maximum	Mean	Maximum	Minimum	Mean	Maximum	Minimum
	years		mm. Hg		cc.	cu.mm./5 cc. of part		No. per minute			cu.mm./5 cc. of part		
39	46	M	200/120	F*	4.2	14.0	38.2	7.8	10	5	12.5	15.0	7.7
				T*	4.0	7.1	22.2	11.0	13	10	5.2	6.2	4.5
				P*	2.0	5.2	17.3	10.0	17	7	9.5	9.7	9.0
40	38	F	220/148	F	3.9	19.8	54.5	11.6	12	6	6.8	7.6	6.4
				T	3.1	5.1	36.9	11.2	12	5	2.9	3.0	2.9
				P	1.2	4.5	20.0	9.8	15	6	1.6	1.6	0.8
44	42	M	198/140	F	7.1	12.3	37.3	8.0	9	6	4.0	4.6	3.5
				T	6.1	5.2	13.8	8.4	10	5	2.1	2.4	1.1
				P	3.0	4.1	10.1	10.2	14	7	8.4	10.0	0.5
57	40	F	190/138	F	3.2	12.4	47.4	7.8	10	6	5.7	7.0	4.7
72	27	F	200/140	F	3.2	14.3	50.8	14.2	18	12	9.1	10.2	6.9
				T	3.9	5.4	19.5	10.2	13	7	2.7	3.9	2.5
74	28	F	184/124	F	3.5	11.1	38.2	9.8	10	9	3.3	5.6	2.2
				T	3.3	7.7	23.0	11.2	14	10	8.3	9.7	6.8
Mean, maximum and minimum for the group				F		14.0	54.5	9.9	18	5	8.2	15.0	2.2
				T		5.1	36.9	10.4	13	5	4.2	9.7	1.1
				P		4.6	20.0	10.0	17	6	6.5	10.0	0.5

\* F = Right index finger tip.

T = Right second toe tip.

P = Postero-superior portion of the right pinna.

The changes in the three parts following deep inspiration were the same as those described in normal subjects, except in the group of senile subjects in whom the decrease in volume of the tips of the fingers and toes was not as great as in the individuals with hypertension or in the normal subjects.

*Alpha waves.* The general configurations and characteristics of these waves in all three groups were similar to those in normal subjects. In *diencephalic hypertension* the mean volume was 13.3 cu. mm. in the finger tip, 8.4 cu. mm.

in the toe tip, and 4.6 cu. mm. in the pinna. The mean frequency was 8.7 per minute in the finger tip, 8.3 per minute in the toe tip, and 11.4 per minute in the pinna (table 1). Patients with diencephalic hypertension did not exhibit type

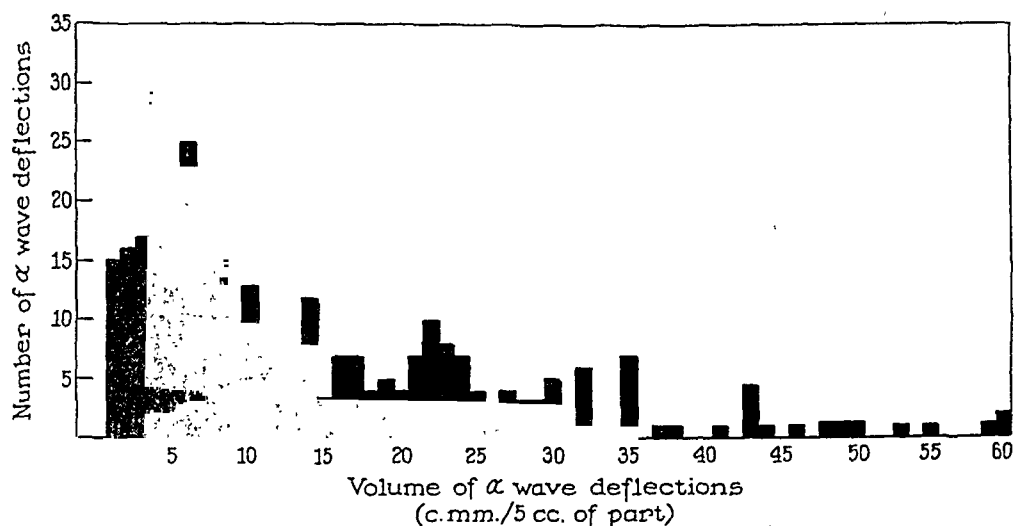


Fig. 1. The distribution is shown of the deflections of the alpha waves during a 15 minute period in 7 patients with "diencephalic" hypertension. Most of the waves are small or of medium size, but there is a wide scattering of large ones.

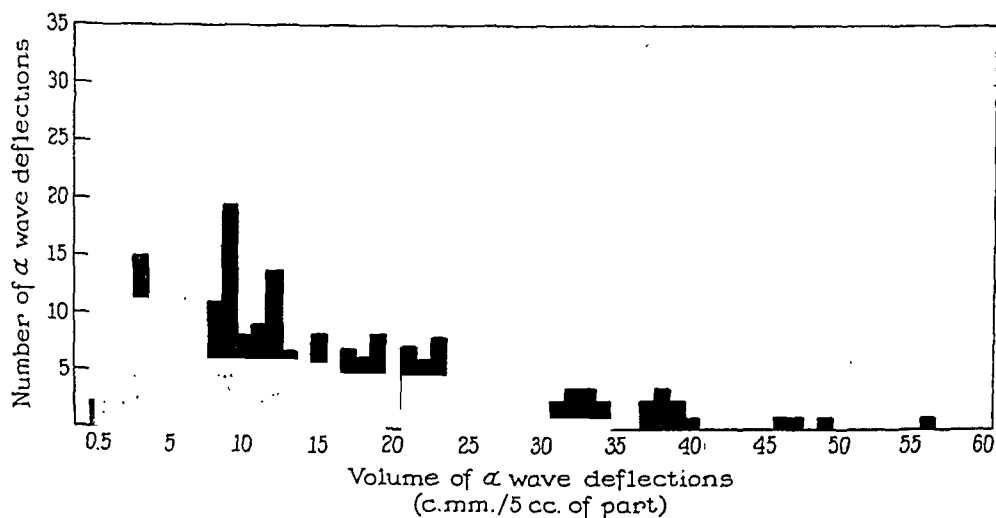


Fig. 2. The distribution is shown of the deflections of the alpha waves during a 15 minute period in 6 patients with "renal" hypertension. The sizes and spread of the waves are similar to those in patients with "diencephalic" hypertension.

I alpha waves. Five exhibited type II, and two, type III (fig. 1). In *renal hypertension* the mean volume was 14.0 cu. mm. in the finger tip, 5.1 cu. mm. in the toe tip, and 4.6 cu. mm. in the pinna. The mean frequency of deflections was 9.9 per minute in the finger tip, 10.5 in the toe tip, and 10.0 in the pinna

(table 2). The six patients with renal hypertension did not exhibit type I waves in the finger tip. Five exhibited type II, and one, type III (fig. 2). In

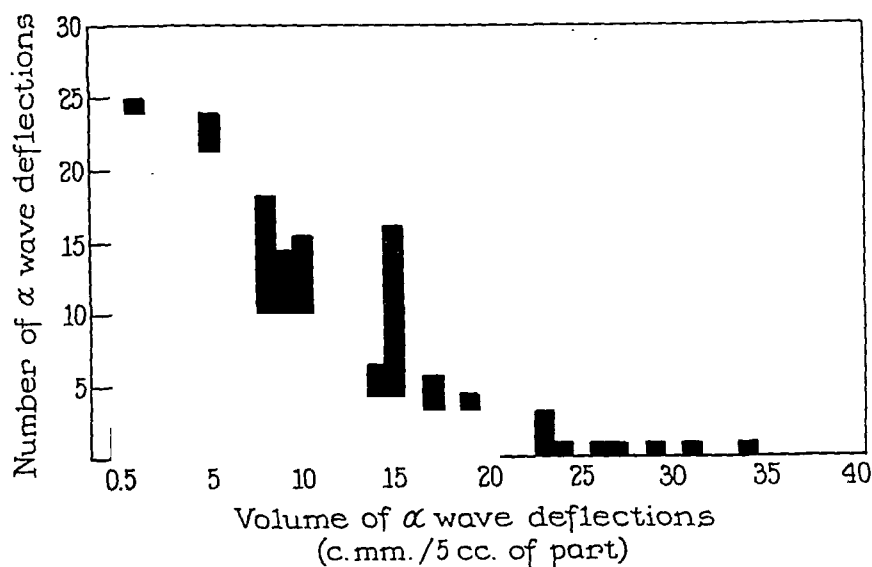


Fig. 3. The distribution is shown of the deflections of the alpha waves during a 15 minute period in 7 senile individuals. The waves are in general much smaller than those in the two groups of hypertensive patients.

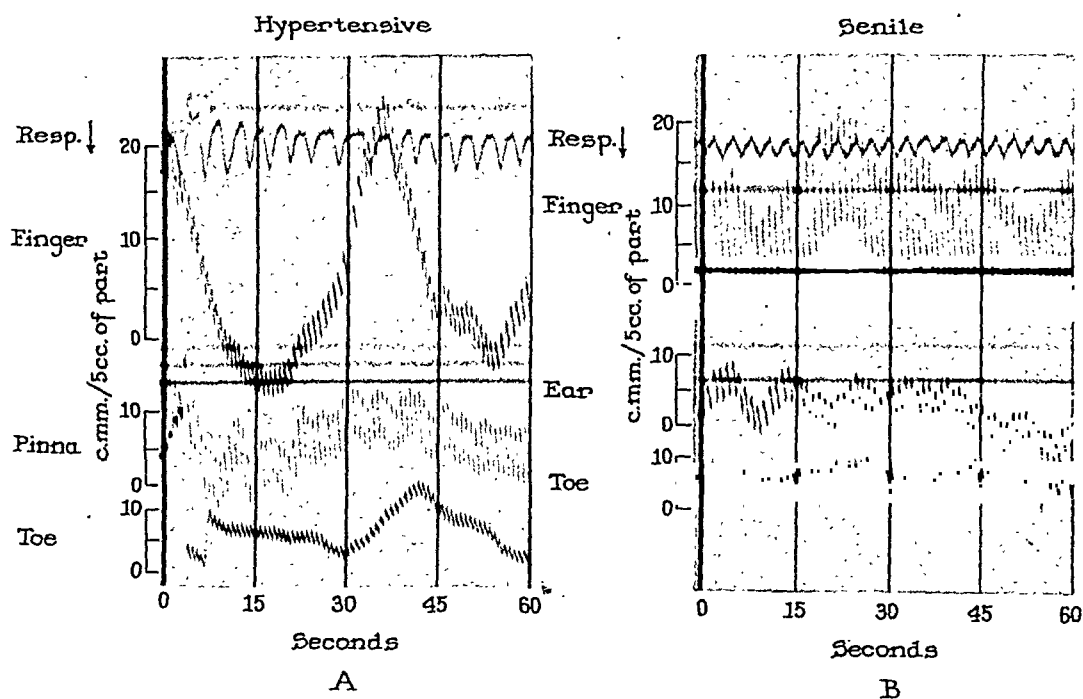


Fig. 4. Original tracings are exhibited of the alpha waves in a patient with hypertension (A) and in a senile subject (B). The waves are much larger and more variable in the patient with hypertension than in the senile one.



*senility* the mean volume was 8.3 cu. mm. in the finger tip, 3.5 cu. mm. in the toe tip, and 4.5 cu. mm. in the pinna. The mean frequency was 7.4 per minute in the finger tip, 5.7 in the toe tip, and 8.9 in the pinna (table 3). The alpha waves of the finger tips of all 7 senile subjects were of type I (fig. 3). Small strips of original representative tracings of alpha waves in a patient with hypertension and in a senile subject (fig. 4) illustrate the fact that the waves

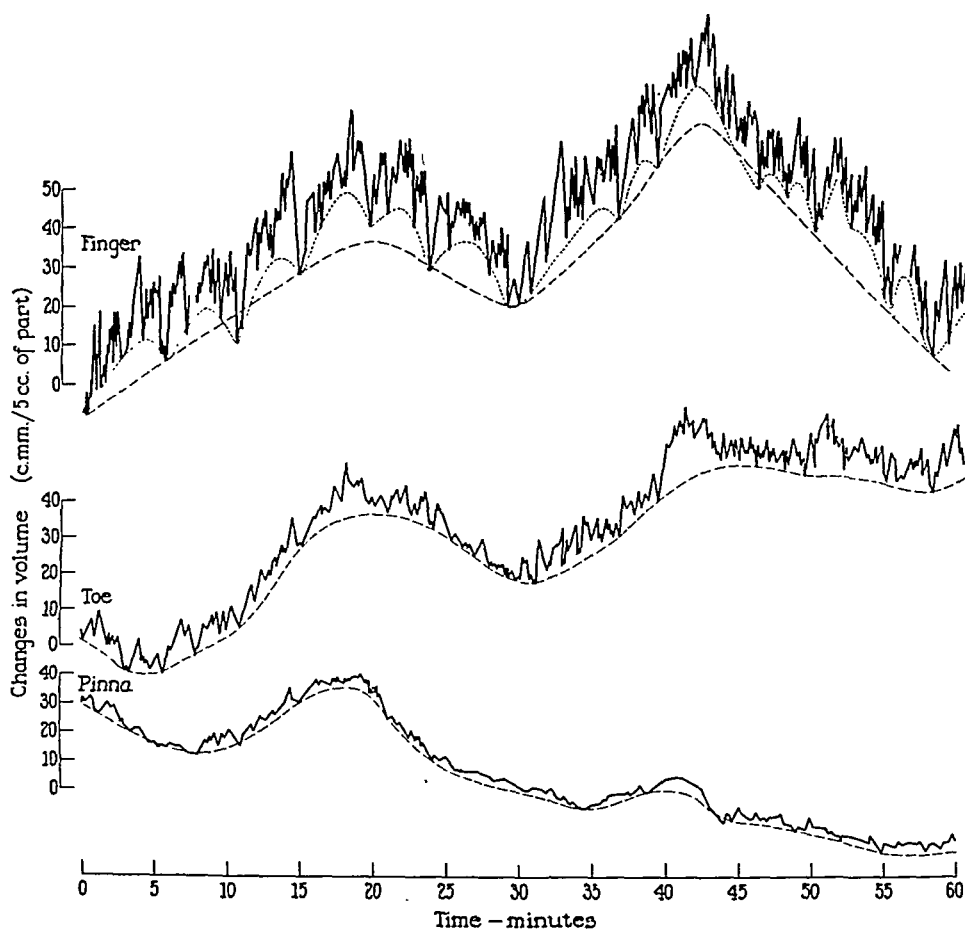


Fig. 5. Here are shown the alpha, beta, and gamma waves in the tips of the finger, the toe, and the pinna of a patient with hypertension. The gamma waves are sharply concordant in the first half. This phenomenon is less conspicuous in the last half of the tracings.

undergo greater variations in volume in patients with hypertension than in senile subjects.

*Beta and gamma waves.* The configurations and characteristics of these waves were essentially the same as in normal individuals. There were no significant differences among the three groups of patients (fig. 5). As in normal subjects it was not unusual for the gamma waves of one part to vary discordantly with those of the others.

DISCUSSION. The mean volumes of the pulse waves of the three groups of patients fell within a narrow range and were of the same order as those of normal subjects. One important exception occurred in the tips of the toes of the senile

TABLE 3  
*Measurements of the pulse and alpha waves in senile subjects*

SUBJECT NO.	AGE	SEX	BLOOD PRESSURE	PART	VOLUME OF PART STUDIED	VOLUME OF THE DEFLECTIONS OF THE ALPHA WAVES		FREQUENCY OF THE DEFLECTIONS OF THE ALPHA WAVES			VOLUME OF THE PULSE WAVES		
						Mean	Maximum	Mean	Maximum	Minimum	Mean	Maximum	Minimum
	years		mm. Hg		cc.	cu.mm./5 cc. of part		No. per minute			cu.mm./5 cc. of part		
27	77	M	148/82	F*	4.9	8.9	27.4	6.0	7	5	4.1	5.1	3.6
				T*	4.7	2.9	15.0	6.0	7	4	1.1	1.3	0.9
				P*	2.1	4.8	12.0	6.0	6	6	2.9	3.6	2.4
35	83	M	160/78	F	5.9	9.0	18.7	7.2	8	5	5.6	6.5	4.1
				T	4.0	3.9	17.3	7.0	9	4	1.9	2.1	1.6
				P	2.5	3.6	8.1	9.4	10	9	3.2	3.6	2.6
47	78	M	105/75	F	4.8	5.5	15.5	8.4	10	4	6.5	7.7	5.8
				T	3.5	3.0	7.0	6.2	10	4	1.3	1.6	1.1
				P	2.1	5.3	14.6	11.6	13	8	9.3	9.8	9.0
51	90	M	165/80	F	6.1	7.4	28.4	8.0	10	5	9.1	9.8	8.6
				T	4.3	3.6	10.4	4.8	6	3	1.3	1.6	0.9
				P	2.3	5.8	16.0	9.0	10	6	6.7	6.9	6.0
55	70	M	98/64	F	5.1	13.3	34.1	7.0	8	5	8.8	10.1	7.3
				T	3.7	6.3	17.8	7.6	10	5	5.5	6.6	3.4
				P	1.8	6.6	12.1	8.4	10	7	2.2	2.4	1.9
68	84	M	136/86	F	5.9	6.0	24.6	7.4	10	6	2.3	2.9	2.1
				T	3.6	2.9	8.4	3.6	6	2	1.4	2.1	1.1
				P	3.3	2.6	7.6	7.8	10	7	4.7	5.8	4.1
91	80	M	140/70	F	4.8	8.0	21.0	7.6	9	5	5.9	6.4	5.4
				T	3.2	1.6	4.8	4.8	5	3	1.4	1.6	1.3
				P	2.6	3.1	9.8	10.4	13	8	3.5	3.9	2.8
Mean, maximum and minimum for the group				F		8.3	34.1	7.4	10	4	6.0	10.1	2.1
				T		3.5	17.8	5.7	10	2	2.0	6.6	0.9
				P		4.5	16.0	8.9	13	6	4.6	9.8	1.9

\*F = Right index finger tip.

T = Right second toe tip:

P = Postero-superior portion of the right pinna.

group in which the volume was one-half of that of other patients and of normal persons. The individual variations of the three parts were great in all the subjects, the maximum values being approximately 5 times the minimum. The

exception to this generalization was again the toe tips of the senile patients in which the variations were very close to the mean. The small pulse waves in the toes of the senile subjects correspond to the accepted belief that the circulation is decreased in the lower extremities of elderly individuals.

The spontaneous waves of patients with diencephalic and renal hypertension were found to be essentially the same in the resting state. Under certain circumstances of stress the peripheral blood vessels may nevertheless behave differently.

In no instance did the alpha waves of the finger tips of hypertensive patients fall into the type I classification (2). Of the 13 patients with hypertension, 10 exhibited type II waves, and three, type III. They were all emotionally unstable and excitable. They were easily disturbed by minor occurrences at home and in the hospital while under observation. They were restless, irritable and anxious during most of the time these observations were conducted. They entered a "relaxed" state with difficulty. To ascertain for what component of the total appearance hypertension on the one hand is responsible and the patient's original psychosomatic constitution on the other would require a more detailed psychometric study.

The alpha waves of the 7 senile subjects were of type I. In senile persons there was less fluctuation in the peripheral blood vessels, probably as part of their general sluggish psychosomatic activity. The extent to which this phenomenon is to be linked with the possibility that emotionally stable and phlegmatic persons live longer is a subject for further study. Marked variations in volume of the peripheral vascular bed may, of course, be inhibited by sclerotic changes. To solve such problems more extensive studies are necessary. In no patients in whom observations were repeated over a period of about one year was a change noticed in the type of their alpha waves.

#### SUMMARY

The spontaneous variations in volume of the peripheral blood vessels of the tip of the right index finger, the tip of the right second toe, and the postero-superior portion of the right pinna were found to be about the same in patients with diencephalic hypertension as in those with renal hypertension. The configurations and other characteristics of the 5 types of rhythm in the blood vessels of these parts were found to be essentially the same as those described as occurring in normal subjects. In no instance were the alpha waves of these patients of type I. Of the 13 patients with hypertension, 10 exhibited type II waves, and 3, type III. All were emotionally unstable and excitable.

In senile subjects the 5 types of rhythmic spontaneous variations were also about the same as in the normal subjects and in the patients with hypertension, with the exception of the volume of the pulse waves of the toes which was smaller. Such small waves are probably due in large part to arteriosclerotic changes. The alpha waves in all of the senile subjects were of the stable variety, type I.

Whether this is due to a sluggish psychosomatic state so well known in senile individuals and also whether individuals who are emotionally stable and not easily excitable live longer is a problem which was not studied.

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# THE DISAPPEARANCE RATE OF TYROSINE FROM THE BLOOD OF DOGS

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The extremely rapid disappearance rate of tyrosine from the blood stream following the intravenous administration was noted by King and Rapport (1). They were unable to find even a trace of it five minutes after the injection of 1 gram and following the injection of 5 grams there was no appreciable increase in blood amino nitrogen and only 5 to 7 per cent was accounted for by the increase in urine amino nitrogen and phenols. These observations suggested that some of the amino acid was stored in some of the body tissues. King, Simmonds and Aisner (2) found only 5 per cent of the acid, however, in the viscera and skeletal muscles 5 minutes after the injection. The content of the blood and tissues, and the urinary excretion of the amino acid and its decomposition products, therefore, accounted for only 12 to 17 per cent of the injected tyrosine. The present investigation was undertaken to ascertain whether or not a uniform disappearance rate could be obtained in normal dogs following intravenous administration of tyrosine, and to study possible factors which would alter this rate of disappearance.

**METHOD.** Healthy dogs were fasted 18 to 24 hours prior to the intravenous administration of 0.2 gram of tyrosine per kilogram of body weight. The tyrosine was administered as a 10 per cent solution in normal sodium hydroxide as described by King and Rapport (1). Blood phenols were measured immediately before and at intervals for 4 hours thereafter on 0.2 cc. samples of blood withdrawn from the marginal ear vein, utilizing the phenol reagent of Folin and Ciocalteu (3). There were twenty-three observations on 6 normal dogs. One dog was fasted for 7 days, and another received in addition to the daily diet, 0.2 gram of tyrosine per kilogram of weight intravenously daily for a week. The disappearance rate was then measured. Hepatic damage was produced in 1 dog by chloroform anesthesia for 2 hours on 2 successive days and the disappearance rate was ascertained on the third day. Chloroform continued to be administered to this dog until the bilirubin content of the blood serum had risen to 7.5 mgm. per cent and extreme edema had developed in the extremities, at which time the tyrosine disappearance rate was again ascertained. The disappearance rate was measured in a 10 kgm. dog 2 hours after the intravenous administration of 10 cc. of India ink. The disappearance rates from the blood and lymph were measured simultaneously in 1 dog under nembutal anesthesia and with the thoracic duct cannulated. The skin, except for that over the head and fore feet, was removed from an anesthetized dog and the disappearance of tyrosine from the blood was then measured.

**RESULTS AND DISCUSSION.** The tyrosine was found to disappear from the blood stream of normal dogs at a surprisingly uniform rate, although the dogs varied in weight from 10 to 20 kgm. The fasting blood tyrosine averaged from 10 to 12 mgm. per cent, reached its height of 80 to 120 mgm. per cent immediately after the injection and declined rapidly for 15 minutes to between 20 and 30 mgm. per cent. Thereafter it declined slowly, but did not reach the fasting level until after 4 hours.

When 2.4 grams of tyrosine were injected into a 12 kgm. dog whose blood volume was assumed to be 1000 cc. the maximum possible blood concentration of the amino acid would have been 240 mgm. per cent. The maximum blood tyrosine level observed was approximately 85 mgm. per cent 1 minute following the injection which required 2 minutes. Approximately 65 per cent of the tyrosine had been withdrawn from the blood stream 3 minutes after beginning of the injection. The fact that the tyrosine decreases rapidly at first from the blood stream and then very slowly for 4 hours or longer suggests that it may be temporarily stored in some tissue or tissues of the body, and then slowly liberated back into the blood stream from which it is withdrawn and metabolized. It does not appear that tyrosine is stored as such for very long. If it were, the depletion and saturation of the hypothetical tyrosine stores of the body in the 2 dogs should have altered the curves.

The tissues most likely to store tyrosine temporarily are the liver, skeletal muscles, reticulo-endothelial system and the skin. Liver damage produced by chloroform anesthesia, however, had no effect upon the rate of disappearance, although in 1 of the 2 experiments the fasting blood phenol level was 100 per cent greater than the normal. The fact that the disappearance rate was not altered following an attempt to block the reticulo-endothelial system suggests that this tissue is not one that temporarily stores the amino acid. It must be remembered, however, that filling these cells with one substance does not satisfy their appetite for another. It appears unlikely from our observation on 1 dog in which the disappearance rate was not altered following the removal of the skin from the body that the skin plays an important rôle in the temporary storage of tyrosine.

The maximum concentration of tyrosine in the thoracic duct lymph did not occur until 2 or 3 minutes after the greatest concentration in the blood and its rate of disappearance paralleled that of the blood stream. This observation suggests that tyrosine is distributed throughout the body fluids within 2 to 3 minutes after intravenous administration.

The observation of King, Simmonds and Aisner (2) that the amino acid is not stored to any great amount in the viscera or skeletal muscles and our observation that the removal of the skin did not alter the disappearance rate suggests that the tyrosine is not stored in any particular organ or tissue, but that it is distributed throughout the body fluids and cells. It appears unlikely that tyrosine is metabolized so rapidly or the urine amino nitrogen and phenols should increase more than that observed by King and Rapport (1). It is probable that the tyrosine is altered in such a manner that it is no longer detectable by procedures

commonly employed. The molecule is very likely not disrupted but it may be combined with other substances.

#### SUMMARY

The disappearance rate of tyrosine from the blood stream following its administration intravenously was found to be rapid and uniform in 23 observations on 6 normal dogs. The rate of disappearance was not altered by fasting, repeated injections of tyrosine, liver damage produced by chloroform poisoning, the previous injection of India ink, or by the removal of the skin. The tyrosine concentration of the lymph reached a maximum about 2 minutes after that of the blood stream and decreased at about the same rate. These observations suggest that tyrosine is distributed throughout the body and that the molecule may be altered so that it is no longer detectable by the usual procedures.

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# THE ABSOLUTE THRESHOLD OF VISION IN CAT AND MAN WITH OBSERVATIONS ON ITS RELATION TO THE OPTIC CORTEX

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Experimental observations on the rat (1, 2), dog (3), cat (4), and monkey (5) have shown that surgical extirpation of the visual areas of the cerebral cortex modifies only in a minor way the ability of these animals to distinguish between lights of different intensity. The just-noticeable-difference threshold of brightness discrimination, as measured in these experiments by presenting two test stimuli of different intensity in a dark surround, was found to be slightly changed after the surgical operations were carried out. The results of these experiments have been interpreted to mean that a unitary neurological process of brightness discrimination exists which possesses only inconsequential relation to the cerebral cortex for these infra-human vertebrates. On the other hand, available data indicate that brightness discrimination in man is determined for the most part by the cortex (6, 7). Accordingly, it has been inferred that the central visual system in man is organized differently from that of lower animals, in that the function here discussed is corticalized in man, but not in infra-human vertebrates.

In order to understand more fully the nature of brightness vision in man and animals, it is necessary to determine further to what extent different aspects and conditions of brightness discrimination, other than those existing in the investigations already mentioned, are related to the functioning of the optic cortex. Some observations on this problem, already reported by one of the authors (8), have shown that in cats surgical removal of the visual cortex abolishes the ability to distinguish between differences in intensity of two test stimuli when they are presented in an illuminated surround. In order to investigate another coördinate aspect of brightness discrimination and determine whether it is dependent upon the normal functioning of the cortical optic centers, observations have been made on the absolute threshold of brightness vision for the cat and the functional value of the cortical projection areas of the retina in its determination.

The absolute brightness threshold in vision is the minimal amount of radiant energy or light which can be observed under complete dark adaptation. As such, it represents the end-point of dark adaptation. For the human eye, this value has been found to be of the order of  $10^{-9}$  ergs (9), or as expressed in photometric units, about  $10^{-7}$  to  $10^{-6}$  millilamberts (10, 11, 12, 13, 14).

We have measured in photometric units the minimal amount of light at absolute threshold for the cat's eye by training the animal to press a pedal—in order to obtain food—when a large panel of flashed-opal glass behind the pedal is illuminated by white light from a tungsten lamp. If the pedal is pressed when



no light appears on the panel, an electric shock is administered. In this situation, the animal learns to press the pedal only when the light stimulus is presented.

The absolute threshold is determined under complete dark adaptation following a procedure in which the stimulus light is gradually reduced in intensity over an extended series of test trials until the ability to respond correctly fails. Thresholds have been obtained for six normal cats, on three of which the measurements were repeated. The absolute threshold of vision in these six animals varied between  $5.8 \times 10^{-8}$  and  $2.4 \times 10^{-7}$  ml., with an average value of  $8.2 \times 10^{-8}$  ml. Threshold values for two human subjects tested under the same conditions were  $5.8 \times 10^{-7}$  ml. Therefore, when measurements are made under the same conditions, the absolute threshold of the cat's eye is, on the average, about one-seventh that of the human eye.

The values obtained for the cat's eye in this experiment represent a lower absolute threshold than any of the photometric values yet reported by other investigators for the human individual. The superiority of the cat's eye over the human eye in detecting small amounts of light is probably not related to a greater absolute sensitivity of the retina of the cat. Rather, the cat's eye, by virtue of its smaller size and greater power of the dioptric system as well as the wide pupillary opening, is sufficiently "faster" than the human eye to explain the differences in the absolute threshold in the two forms. Comparison between the absolute brightness threshold of the cat's eye and that of other infra-human animals which have been tested indicates that the cat is unequalled in its ability to detect small amounts of light under dark adaptation.

Surgical operations were performed on two of these cats in which the visual areas of the cerebral cortex were partly removed in one animal (animal 1) and completely removed in the other (animal 2). In animal 1, all of the visual cortex was extirpated except for a strip of the posterior mesial surface of the gyrus splenialis on the right side. This gyrus constitutes the mesial aspect of the area striata or optic cortex of the cat. Degeneration of the external geniculate body in this animal extended throughout the nucleus except for scattered ganglion cells in the inferior medial region of the body. The optic cortex of animal 2, as well as the tissue of the gyri bordering the lateral and splenial gyri, was completely destroyed by the operation.

After recovery from the operations, the animals were again trained and tested for the absolute threshold. The results are presented in table 1, which summarizes the preoperative and postoperative thresholds obtained for these two cats. Animal 1 displayed a fivefold increase in the magnitude of the threshold after operation, animal 2 a five-hundredfold increase. The results on both animals seem to provide clear evidence that the absolute brightness threshold is directly dependent upon the normal functioning of the optic cortex. Since these findings have been obtained, confirming data have been secured by Kapauf (15) in terms of the disappearance of flicker discrimination at low brightnesses and by Mead (16) in regard to differential brightness discrimination at

low brightnesses. Both Kappauf and Mead obtained results on cats which had suffered complete removal of the visual cortex, and found that the required discriminations disappeared at a level of brightness comparable to the post-operative absolute threshold of animal 2, undoubtedly because the absolute level of sensitivity of the animals' eyes had been reached.

We conclude that the optic cortex in animals plays a significant rôle in certain aspects of brightness discrimination. It is apparently directly involved in defining the limits of vision at absolute threshold, and, as shown in a previous study cited, the cortex maintains the ability of animals to distinguish brightness differences in illuminated surrounds.

Additional information is necessary before it will be possible to suggest any general theory of the conditions under which the cortex plays an important rôle in brightness vision. But it is certain that this capacity is partly corticalized, so to speak, in the cat and in other infra-human animals, and therefore the clear-cut distinction between man and other vertebrates in this regard cannot be maintained. Since, for the cat, the functional value of the cortex in bright-

TABLE 1

ANIMAL	TEST	THRESHOLD BRIGHTNESS	THRESHOLD INCREASED
		<i>ml.</i>	
1	Preoperative	$1.2 \times 10^{-7}$	5 times
	Postoperative	$5.8 \times 10^{-7}$	
2	Preoperative	$0.58 \times 10^{-7}$	500 times
	Postoperative	$0.29 \times 10^{-4}$	

ness vision depends on specific aspects of the stimulus conditions, the possibility arises that more thorough investigation of human individuals lacking the optic cortex might reveal some residual brightness vision mediated by subcortical centers, for it is evident that such observations as have been made on man have not been sufficiently extensive to say whether or not the visual cortex is indispensable for brightness vision.

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# CHEMICAL COMPOSITION OF HUMAN SEMEN AND OF THE SECRETIONS OF THE PROSTATE AND SEMINAL VESICLES<sup>1</sup>

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The chemical nature of human semen has been incompletely described and quantitative data are lacking for important electrolytes as well as the proteins of this fluid. The seminal plasma of man is largely composed of the secretions of the prostate and the seminal vesicles. Little is known of the function of these accessory sex structures and few chemical data have been reported on the external secretions of these glands which are not known to have an endocrine secretion. The present study was conducted to determine chemical values for the principal electrolytes and proteins of normal human semen and of the secretions which are its chief constituents. The effect of varying the frequency of ejaculation on the chemistry of semen and the relationships of androgen injections were also studied.

**RESULTS OF PREVIOUS INVESTIGATIONS.** Chemical studies of the semen of the boar have been made by McKenzie, Miller and Baugess (1938); of the stallion by Davis and Cole (1939); of the goby (*Gillichthys mirabilis*) by Young and Fox (1937); and of the dog by Huggins, Masina, Eichelberger and Wharton (1939). The earlier literature on quantitative studies of human semen has been summarized by Scherstén (1936) and Zagami (1939).

*Human semen.* Normal human semen has a pH varying from 7.05 to 7.41 and contains large amounts of reducing substances, glucose, calcium, acid soluble phosphorus and relatively small amounts of chloride; the findings are not appreciably changed by bilateral ligation of the vas deferens (Huggins and Johnson, 1933). Goldblatt (1935) studied the phosphorus compounds and observed a high urea and lactic acid content in semen; cholesterol is somewhat lower than in blood plasma. Messer and Almquist (1937) obtained a mean value of pH 7.25 for fresh semen. Hotchkiss, Brunner and Grenley (1938) found average glucose values of 306 mgm. per cent for this fluid. Harrison (1933) obtained values for spermine phosphate, 154 to 233 mgm. for 100 cc. Scherstén (1936) reported a large content of citric acid in human semen; none was found in the seminal vesicle and the chief source of citrate was the prostate. Semen is richer than plasma in ascorbic acid (Berg, Huggins and Hodges, 1941).

*Human prostatic fluid.* Analysis of combined prostatic-vesicular secretions obtained by rectal massage were reported by McCarthy et al. (1928); since variable concentrations of these secretions were present in the fluids which these authors analyzed it is difficult to compare their results with the data of

<sup>1</sup> This investigation was aided by a grant from the National Committee on Maternal Health.

others. Huggins and Johnson (1933) found that prostatic fluid contained only small amounts of reducing substances and phosphate. Moore, Miller and McLellan (1941) obtained total cholesterol values of 314 and 332 mgm. per cent in the prostatic fluid of normal men.

*Human seminal vesicle secretions.* Huggins and Johnson (1933) observed high concentrations of glucose, reducing substances, acid soluble P, and non-protein nitrogen in this fluid. Berg et al. (1941) found that this structure concentrated ascorbic acid.

**METHODS.** Chemical studies were made on the prostatic fluid of 34 healthy men and on the seminal vesicle secretion of 7 men; these fluids were obtained by digital massage of the appropriate structure through the rectum and the first two drops of secretion appearing at the urethral meatus were discarded to minimize contamination with urine. It is difficult to obtain subjects from whom large amounts of vesicular secretion can be obtained. The semen of 56 men in good health was collected by ejaculation into a glass tube; analysis was begun after liquefaction of the semen had occurred, usually about 20 minutes after ejaculation. Microscopic examination was always done and specimens exceeding the normal count of leukocytes were discarded. The semen was well mixed by agitating the containers and for the electrolyte studies it was centrifuged at 3000 r.p.m. for 10 minutes, the analyses being performed on the supernatant seminal serum. For studies of pH and bicarbonate the fluids were delivered directly into tubes filled with oil.

*Chemical methods.* Water was determined by drying known weights of the fluid to constant weight; pH by the glass electrode; total CO<sub>2</sub> by the method of Van Slyke and Neill (1924); chloride by the method of Van Slyke (1923) as modified by Wilson and Ball (1928); sodium by the method of Butler and Tuthill (1931) as modified by Eichelberger (1938), potassium by the method of Tenery and Anderson (1940) except that pyrex tubes were used instead of quartz.

Total nitrogen and in some cases non-protein nitrogen were determined by the micro-Kjeldahl technique of Goebel (1932). Non-protein nitrogen was also determined after the proteins had been precipitated and centrifuged away, by digestion and Nesslerization of the supernatant. The proteins were estimated by multiplying by 6.25 the total nitrogen corrected for NPN. Difficulty was encountered in securing satisfactory precipitants for seminal proteins and obviously incomplete precipitation attended the use of 100 per cent ethyl alcohol, dioxane, 10 per cent trichloroacetic acid and the tungstate-sulfuric acid mixture of Folin and Wu (1919). More satisfactory results were obtained by acetone precipitation after acidification according to the method of Folin and Denis (1923); NPN was determined on the supernatant and following further washing with acetone, the precipitate was weighed. Globulins were determined by the method of Howe (1921).

The amount of protein coagulated by heat was determined as follows: a measured amount of semen in a graduated centrifuge tube was acidified to pH 5.5 with acetic acid and placed in a water bath at 100°C. for 30 minutes,

evaporation being prevented by inserting a second centrifuge tube in the mouth of that containing the sample; after centrifuging, an aliquot of the heated supernatant fluid was taken for nitrogen determination. Dialysis experiments were done by placing semen in 0.5 cc. amounts in a cellulose bag immersed in a large jar of distilled water at 37°C. Toluol was added to the sample as a preservative. The water was changed many times and dialysis was carried out for three days. As a control, blood serum was dialyzed in similar bags in each case. The amount

TABLE 1

*Chemical composition of normal semen, prostatic fluid and seminal vesicle secretion*

	SEMEN				PROSTATIC FLUID				SEMINAL VESICLE FLUID			
	No.	High	Low	Average	No.	High	Low	Average	No.	High	Low	Average
Values per liter of fluid												
pH.....	9	7.36	6.9	7.19	3	6.6	6.33	6.45	2	7.32	7.26	7.29
Water, gm.....	13	944	891	918	5	936	927	932	2	900	880	890
Sodium, mM.....	14	133	100	117	5	158	149	153	1	103		
Potassium, mM.....	12	27.4	17	22.9	6	61.4	28.7	48.3	2	21.2	14.3	17.8
Calcium, mM.....	3	7.15	5.3	6.22	3	32.7	28.7	30.2				
Total CO <sub>2</sub> , mM.....	7	33.2	19.2	24	3	5.4	3.1	4.2				
Chloride, mM.....	31	57.3	28.3	42.8	8	46.1	34.8	38.1				
Acid soluble phosphorus*	8	32.3	17.2	23.8	16	1.77	0.65	1.09	7	19.8	9.65	14.7
Specific gravity.....	6	1039	1031	1035	14	1027	1018	1022	2	1038	1036	1037

Values per 100 cc. of fluid

Total N, mgm.....	34	1225	560	913	14	511	295	416	3	1343	1233	1284
Non protein N, mgm.....	12	130	73	96	6	90	30	53.6	1			99
Total protein by difference, gm.....	12	6.85	3.29	4.50	6	2.93	1.66	2.17	1			7.78
Total protein, gravimetric, gm.....	11	7.74	4.30	5.80	2	2.64	2.46	2.55	1			9.04
Globulins, gm.....	6	2.43	0.76	1.20								
Glucose,* mgm.....	6	369	203	295	12	48	Trace	16.4	5	625	275	390
Ascorbic acid,** mgm.....	9			12.8	19			0.54	9			4.66
Inorganic P,† mgm.....				45								
Spermine P,† mgm.....				22.5								
Urea,† mgm.....				72								
Lactic acid,† mgm.....				95								
Cholesterol,† mgm.....				80		618	865					

\* Data of Huggins and Johnson (1933).

\*\* Data of Berg, Huggins and Johnson (1941).

† Data of Goldblatt (1935).

§ Data of Moore, Miller and McLellan (1941).

of protein remaining in the bag was estimated from nitrogen determination following digestion of the entire bag and its contents.

RESULTS AND DISCUSSION. Semen was consistently slightly more acid than blood plasma and the acidity of the sample increased when ejaculation occurred at a short interval after the preceding sample was obtained. Sodium, chloride and total protein are less in amount than corresponding values of blood plasma (table 1). The content of calcium, potassium, non-protein nitrogen, acid soluble phosphorus, glucose and reducing substances including ascorbic acid are considerably increased above plasma levels.

*Prostatic secretion.* Compared with semen, this fluid contains more water, has a lower specific gravity and a lower pH (table 1). It also contains less chloride and total proteins while the content of glucose and bicarbonate is low. The prostatic fluid is rich in inorganic cations, notably sodium, potassium and calcium; not enough inorganic anions were found to balance the cations so as to account for the observed pH of 6.45. It is possible that the proteins being of smaller molecular size than the plasma proteins are also ionized to a greater extent and that lactate, citrate and other organic ions make up the anion deficiency. In comparing the findings in semen with those of the accessory glands it should be noted that semen is a fluid produced at least in part by nerve stimulation, while the secretion of the accessory glands was obtained during a resting period; Berg, Huggins and Hodges (1941) in the dog showed that the resting prostatic fluid obtained without adventitious stimulation differed in composition from that produced following pilocarpine injection.

TABLE 2  
*Dialysis of semen and serum through cellulose membranes*  
Time of dialysis, 72 hours

	TOTAL N	NON-PROTEIN N	TOTAL PROTEIN, ORIGINAL	TOTAL PROTEIN AFTER DIALYSIS	PROTEIN DIALYZED
Semen					
	mgm./100 cc.	mgm./100 cc.	gm./100 cc.	gm./100 cc.	per cent
K K	676	113	3.52	1.46	58.5
S Z	780	80	4.38	1.46	66
Serum					
K K	1118	28	6.81	6.81	0
W S	1170	38	7.08	7.06	0

*Seminal vesicle secretion.* When human semen stands in a glass tube following liquefaction, for many minutes the characteristic yellow translucent seminal vesicle secretion settles to the bottom of the tube and the prostatic fluid is layered above it. Vesicular secretion was found to have a high specific gravity, contained less water and was rich in proteins, NPN, acid soluble phosphorus, glucose and potassium (table 1).

*The proteins of semen.* Evidence was obtained from dialysis and heat coagulation that the seminal proteins are in large part derived proteins. In the dialysis experiments, 41 to 68 per cent (average 60 per cent) of the seminal proteins passed through a cellulose membrane in three days, while none of the serum proteins dialyzed simultaneously passed through this membrane (table 2).

On heating specimens of semen, either untreated or acidified with acetic acid to pH 5.5 and heated in a bath containing boiling water, only a trace of protein coagulated in four instances and 1.6 to 18 per cent of the total protein coagulated in five cases. Posner (1888) referred to the non-coagulable protein of semen as

propeptone and Goldblatt (1936) made qualitative tests which likewise supported the view that proteoses are present in semen. The present observations show that much of the seminal proteins are proteoses. It is of interest that Young and Fox (1937) observed that much of the seminal vesicle protein of the goby is secondary proteose. The globulin content of semen on average was 1.5 gram per 100 cc. and accounted for 21.4 to 40 per cent (average 29.4 per cent) of the total seminal proteins.

It was observed that gravimetric values obtained for proteins by acetone precipitation in acid solutions were consistently higher (20 to 40 per cent) than the proteins estimated by nitrogen difference (total N-NPN  $\times$  6.25). We believe that the latter method is more accurate since complex carbohydrate molecules such as mucins are known to be present in semen and precipitated by acetone.

TABLE 3

*The similarity of ejaculates collected at a constant interval*

S. Z. age 27 years; the interval since last ejaculation in each case is 60 hours.

DAY	CHLORIDE	TOTAL N	NON-PROTEIN N	PROTEIN
	<i>mM/liter</i>	<i>mgm./100 cc.</i>	<i>mgm./100 cc.</i>	<i>gm./100 cc.</i>
1	44.7	838	113	4.54
54	50.8		92.4	
61	45.6	780	92	4.22
68	44.5	820		
75	47	767	99.6	4.18
82	46.1	780	99.6	4.25
89	48.2	780	80	4.37
96	46.2	780	99.6	4.25
103	45.9	780	99.6	4.25

*Factors affecting the chemical composition of semen.* A similarity was observed frequently in chloride, water and protein content in samples of semen from a single individual. When semen from an individual was collected at a constant interval after the preceding ejaculation, the findings were similar over many months (table 3). Usually greater variations occurred between the semen of different individuals than between samples of one person. The factors found to modify seminal composition were great frequency of ejaculation and the androgen status.

The effect of frequency of ejaculation was studied in three men; after a control period of ejaculation at 3 to 6 day intervals semen was collected 5 times in two days. Typical results are shown in table 4; seminal samples obtained less than 24 hours after a previous ejaculation had an increased water and a decreased protein content, especially those obtained after a 6 to 14 hour interval. From the sample of 56 normal men obtained at random but known intervals, it appeared that the maximum protein content appeared between 48-72 hours after the preceding ejaculations and further continence did not result in a more con-



centrated fluid; occasionally recovery of concentration occurred between 24-48 hours.

The effect of androgen administration was studied in a man, A. M., age 26 years, who was normal except for hypogonadism due to undescended testes; prior to androgen administration this man had infantile genitalia, was beardless and while he had had numerous penile excretions there was no history of ejaculation. The androgen status of this man has been reported by Kenyon and co-workers (1937). During 36 weeks 52 nitrogen studies were made on his semen and in this period testosterone propionate, 25 mgm. daily, was injected in four courses lasting three to four weeks with intervening periods free from androgen. Semen was collected at least once each week usually 48 hours after a previous ejaculation. The maximum total nitrogen value, 1002 mgm. in 100 cc., was obtained during the course of androgen injections. There was a progressive decrease in this value each week in the androgen free periods, to 234 mgm. 84 days following cessation of testosterone injections. The chloride concentration in the periods when androgen was not injected on average was 94 millimols per

TABLE 4

*The effect of frequency of ejaculation on seminal nitrogens*

A. M.: age 26 years; the ejaculations are consecutive

TIME SINCE LAST EJACULATION	TOTAL N	NON-PROTEIN N	PROTEIN
<i>hours</i>	<i>mgm./100 cc.</i>	<i>mgm./100 cc.</i>	<i>gm./100 cc.</i>
35	975	72	5.64
40	963	72	5.57
14	555	60	3.19
10	555	60	3.19
24	848	72	4.95

liter, a figure much higher than the average for normal semen; during androgen administration chloride decreased to 53 millimols. Whenever protein increased in amount chloride decreased in the semen of this man.

#### SUMMARY

The proteins and principal electrolytes of semen and of prostatic and vesicular secretions were quantitatively studied in 56 normal men.

In semen, the amounts of calcium, potassium, glucose, organic phosphate and non-protein nitrogen are considerably increased above their respective concentrations in plasma; sodium and total protein and chloride concentrations are less than similar plasma values.

Human prostatic fluid has a lower specific gravity and pH (6.45) and contains more water than semen. Inorganic cations greatly exceed inorganic anions. Compared with blood plasma and semen this fluid has much less protein and bicarbonate but is richer in sodium, potassium and calcium. The seminal vesicle secretion is heavy, slightly alkaline and contains more protein than semen or prostatic fluid.

The seminal proteins are largely proteoses; on average 60 per cent of the proteins dialyzed through cellulose membranes impermeable to blood serum proteins, and only traces to 18 per cent of the proteins were coagulated in a bath of boiling water. The average globulin content of semen was 1.2 grams in 100 cc. accounting for 21.4 to 40 per cent (average 29.4 per cent) of the total seminal proteins. The protein concentration is decreased in subnormal androgen states and is increased by androgen administration. The proteins of semen are also decreased by very frequent ejaculation, the time for recovery to normal concentrations varying between 24 and 72 hours. The chemical composition is similar in repeated semen samples obtained from a single individual at a standard time since the previous ejaculation.

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# HISTOCHEMICAL CHANGES IN THE MYOCARDIUM OF DOGS FOLLOWING EXPERIMENTAL TEMPORARY CORONARY ARTERIAL OCCLUSION<sup>1</sup>

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The effects of ischemia on muscle in general, and on the myocardium in particular, have been of continual interest to investigators of muscle physiology and pathology. The electrocardiographic and morphological changes which follow brief periods of coronary arterial occlusion have already been reported (2) (3). The present investigation is concerned primarily with the *chemical* changes in the myocardium which result from the temporary occlusion of a coronary artery.

The data have been interpreted *histochemically*. The chemical alterations have been considered as the reflections of changes in either *a*, the proportions of extracellular and intracellular tissue, or *b*, the composition of either the extra- or intracellular tissue. Other investigators have made similar morphological interpretations of chemical data in connection with other problems (4) (5) (6). Such treatment of the data seems particularly desirable in the present instance because of the great difference between the composition of the muscle fibers and of the surrounding tissue. Because of this difference, a change in the relative amounts of these two fractions of the myocardium would cause marked changes in the chemical composition of the tissue as a whole. By emphasizing the two separate fractions of the myocardium rather than the whole tissue mixture, a more intimate picture is perhaps obtained of the chemical events which follow temporary myocardial ischemia.

Chemical changes in the myocardium have been observed by previous investigators following experimental coronary occlusion. These changes consist of an increase in lactic acid and water, and a decrease in glycogen, total phosphorus and creatine (7) (8) (9) (10). Most of these observations have been made following permanent arterial occlusion or after markedly prolonged temporary occlusion. We have investigated the consequences of relatively short-term *temporary*, rather than permanent ischemia to the heart for the following reasons. Temporary relative ischemia is believed to be frequently responsible for myocardial necrosis in the human heart (3). Furthermore, as an aid toward understanding the consequences of ischemia, temporary arterial occlusion offers certain advantages over permanent occlusion. With the latter, there is little opportunity for exchange of materials between the killed or injured fibers and the rest of the body, since the affected region is shut off from the blood stream. As a consequence, substances, perhaps released by injury from the fibers, cannot

<sup>1</sup> A preliminary report of this study has been reported elsewhere (1).

escape from the damaged region, nor can other substances in the blood stream gain access to this region. On the other hand, in the case of a temporary occlusion, after the circulation is restored, the fibers may equilibrate via the blood stream with other parts of the body.

The difference in the results of permanent and of temporary occlusion is well illustrated in the work of Tennant *et al.* (7). These workers observed that permanent occlusion caused a marked rise in lactic acid and a fall in glycogen concentration in the cardiac muscle of the dog. However, following temporary occlusions of 2 and 8 hours, the glycogen fell as before, but no rise in lactic acid was observed. The lactic acid liberated had apparently been washed away following the restoration of the blood supply.

It was previously reported (3) that following temporary experimental occlusion of the left circumflex coronary artery in dogs, areas of necrosis developed in the myocardium, provided that the occlusion lasted longer than 20 minutes. The histological changes generally were not apparent during the first 24 hours. Electrocardiographic changes likewise resulted from the temporary arterial occlusions, but, in contrast to the histological findings, were apparent soon after the operation, and were frequently observed following occlusions of less than 20 minutes' duration.

It was considered possible that chemical examination of the myocardium following such temporary ischemia might reveal changes not recognizable histologically, and would likewise serve to supplement the histological data; to this end, the present histochemical studies were made.

The results indicate that chemical changes are present before morphological alterations are visible. One of the earliest histochemical changes resulting from temporary coronary occlusion appears to be an increase in the amount of extracellular fluid.

**MATERIAL AND METHODS.** *Operative procedure.* Occlusion of the left circumflex coronary artery was performed on 10 adult dogs (under nembutal anesthesia). The operative procedure has been described in detail in a previous communication (3). The artery was dissected free and occluded for 45 minutes or less by applying tension on a ligature passed beneath it. The approximate point of arterial occlusion and the myocardial area thereby deprived of its blood supply may be seen in figure 1.

In 6 control animals, identical operations were performed except that the ligature under the coronary artery remained slack, thereby causing no interference with the blood flow. The purpose of making sham occlusions was to determine whether the operation itself or the manipulation of the coronary artery, prior to the occlusion, might have had any effects upon the myocardium. These hearts also served to show the amount of variation in the chemical composition between different regions of individual hearts.

When the desired time had elapsed after the operation, the animals were again anesthetized with nembutal, and the hearts were removed while still beating.

In both control and experimental animals, samples of tissue were taken not

only from parts of the heart supplied by the left circumflex artery, but also from other regions. Thus, by obtaining tissue from control regions of the same hearts, the effects of individual variations between different hearts were eliminated.

Six samples of cardiac muscle were taken from each heart. Three "affected" samples were selected from regions of the left ventricle, right ventricle, and septum supplied by the artery that had been occluded, and three control samples were removed from regions supplied by intact arteries.

The tissue samples for chemical and histological examination were taken as soon as the beating heart was removed. Samples for electrolyte analyses were stored in stoppered weighing bottles in the ice box; samples for glycogen analysis were weighed quickly on removal and placed immediately in Zenker's solu-

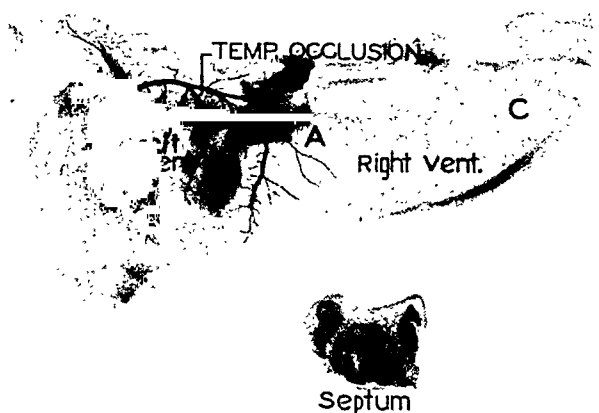


Fig. 1. X-ray photograph of the opened heart with injected left circumflex artery showing the point of temporary occlusion and the regions affected (A) and unaffected (C) which were taken for study.

tion. The speed of handling was designed to minimize loss of water or glycogen from the tissues.

Blood samples were obtained from the superior vena cava. Most of the blood taken was allowed to clot under oil, and the serum separated. One sample of blood was oxalated and preserved as a standard for calculating the amount of blood in the cardiac muscle by quantitative spectrophotometric analyses.

The serum was analyzed for water, chloride, and sodium. The cardiac muscle samples were analyzed in triplicate for chloride, in duplicate for sodium and potassium, and single determinations were made for water, fat, and blood. In some cases, glycogen was also measured.

*Serum analyses.* The serum chloride was measured essentially according to the Wilson and Ball modification of the Van Slyke procedure (11). The serum sodium was measured as described by Hald (12), except that a centrifuge technique was employed as outlined below for tissue. The serum water content was determined by drying 1 cc. portions at 100°C. overnight.

*Tissue analyses.* As soon as the samples of myocardium were chilled to the

ice-box temperature, they were trimmed free of epicardial fat and the tag ends of the chordae tendineae. By working in a cold-room, water loss during the trimming operation was kept to a minimum. The individual tissue samples were reduced to a finely divided state, suitable for the taking of aliquots, by grinding in a mortar with liquid air. Samples were then weighed for subsequent analyses.

*Chloride analyses* were performed on 0.3 to 0.5 gram samples as described by Hastings and Eichelberger (6).

*Sodium and potassium* were determined on a 10 per cent trichloroacetic acid extract of a single 1 to 5 gram sample of tissue. Aliquots of this trichloroacetic acid extract were evaporated with 0.5 cc. of 10 N sulfuric acid per gram of tissue, and wet ashed in pyrex tubes with the repeated addition of small amounts of nitric acid. Following the wet ashing, phosphate was removed by adjusting the pH to 5.0 with redistilled ammonium hydroxide.

Aliquots of the phosphate-free solution were heated in platinum crucibles to 500–550°C. in a muffle furnace, and the potassium in the ash was determined gravimetrically as the chloroplatinate. The conditions employed for precipitation of the potassium chloroplatinate were approximately those described by Hald (12). However, the precipitate was isolated by centrifuging and washing in a tube with a pointed tip, rather than by filtration.

Sodium was determined on the supernatant fluid and washings which were siphoned from the tube containing the potassium chloroplatinate precipitate into another (5 cc.) centrifuge tube. These alcoholic washings, containing the sodium, were evaporated by placing the centrifuge tube in a water-bath at 50° and directing a jet of air into the tube. When not quite dry, the tube was cooled and 4 cc. of Barber-Koltoff uranyl-zinc-acetate reagent was added. Thorough stirring was accomplished by twirling in the tube a slender glass rod provided with a sine-wave-shaped bend a few centimeters above the lower end. After standing 30 minutes, the tube was centrifuged, and the supernatant fluid siphoned off. Washing of the precipitate was accomplished by stirring up the precipitate with 4 cc. of a wash solution and recentrifuging. The precipitate was washed twice, once with each of two different washes both of which had been saturated with sodium zinc uranyl acetate. The first wash consisted of 78 volumes of acetic acid, 20 volumes of 95 per cent alcohol, and 2 volumes of Koltoff reagent. The second wash contained 95 volumes of 95 per cent alcohol, and 5 volumes of acetic acid. During hot, humid weather, these operations were carried out in a cold-room. After drying the tubes overnight at 38°C., they were weighed on a microbalance. Duplicate analyses checked within 1 or 2 per cent, and known solutions gave values within 1 per cent of the actual content.<sup>2</sup>

<sup>2</sup> A slight improvement was later made in these washes. The first wash was changed to glacial acetic acid plus 2 per cent by volume of the Koltoff reagent; and the second wash consisted of 10 volumes of acetic acid, 80 volumes of 95 per cent alcohol, and 10 volumes of the first wash, both washes being saturated with triple salt just before use. The second wash was prepared freshly each time it was used. To insure the removal of the last traces of solvent, it was found expedient to heat the tubes for 1 hour at 100° before weighing.

*Fat and water* concentrations were determined as described by Hastings and Eichelberger (6). The residual *blood* in the tissue was estimated either by comparison of the animal's own blood with an aqueous tissue extract after conversion to acid hematin (6), or by measurement of the hemoglobin itself in blood and tissue by the use of the spectrophotometer. The difference in the position of the myoglobin and hemoglobin absorption bands permitted correction of these figures for myoglobin in a manner suggested by Watson (13).

The *glycogen* measurements were performed according to Good, Kramer, and Somogyi (14), with slight modifications.

*Calculations.* All tissue data were calculated on a blood-free, fat-free basis. From the sodium and chloride values the amount of the extracellular fluid was calculated as detailed by Hastings and Eichelberger (6). The calculations were somewhat modified through the use of the assumption that the extracellular fluid of the heart muscle contains 4 per cent protein instead of none as had been assumed in the case of skeletal muscle. The assumption of this concentration of protein in the extracellular fluid is in keeping with the observation of Drinker *et al.* (15) on the composition of cardiac lymph. As a consequence, the Gibbs-Donnan ratio for the distribution of ions between serum and extracellular fluid was estimated as 0.98 instead of 0.95. The grams of total extracellular tissue per kilogram,  $E$ , was estimated as the sum of the extracellular fluid and the collagen, which was assumed to be 10 grams per kilogram in the muscle of the left ventricle and septum, and 14 grams per kilogram in the muscle of the right ventricle (16).

The symbols  $E_{Cl}$  and  $E_{Na}$ , are used to signify whether the amount of extracellular tissue per kilogram of muscle was calculated from the chloride or from the sodium concentrations, in one case the chloride and in the other case the sodium being assumed to occupy an exclusively extracellular position. The relative mass of the intracellular compartment,  $C$ , was determined by subtracting the average of  $E_{Cl}$  and  $E_{Na}$  from 1000.

The concentration of water and potassium in the fibers,  $(H_2O)_C$ , and  $(K)_C$ , was likewise calculated, after deducting the extracellular water and potassium.

These histochemical calculations have been made not only for the control samples of cardiac muscle, but also for the samples that were removed from the regions of the heart that had suffered a period of ischemia. It is, of course, possible that as a consequence of damage to the fibers, chloride or sodium or both might have entered the muscle fibers, in which case these ions would no longer serve as accurate measures of the amount of extracellular fluid. Fortunately, the results of the calculations themselves give some evidence as to the validity of the assumptions used in making the calculations. This point will be discussed together with the data.

**RESULTS.** The data are presented as  $a$ , the original analytical values for water, chloride, sodium, potassium, and glycogen; and  $b$ , the derived histochemical values obtained from these primary data.

For each part of the myocardium—i.e., left ventricle, septum, and right ventricle,—values are given side by side in the tables for both the region mainly

supplied by the artery occluded and for a region not directly supplied by this artery. These regions are called "affected" and "control" respectively. The completeness with which the affected portion was supplied by the occluded artery is undoubtedly different in each of the 3 parts of the myocardium, and

TABLE 1  
*Analysis of control hearts*

(H<sub>2</sub>O)<sub>c</sub>, (K)<sub>c</sub>, (Gly)<sub>c</sub> based on a kilogram of fibers.

Other data based on a kilogram of fat-free, blood-free tissue.

	TISSUE	H <sub>2</sub> O	Cl	Na	K	GLY- COGEN	E <sub>Cl</sub>	E <sub>Na</sub>	C	(H <sub>2</sub> O) <sub>c</sub>	(K) <sub>c</sub>	(Gly) <sub>c</sub>
		grams	mEq.	mEq.	mEq.	grams	grams	grams	grams	grams	mEq.	gram
Dog 1, 4.5 hours post operatively	Serum	921.9	114.8	143.0								
	L.V. Control	780.7	24.0	30.0	94.1	9.4	209	217	787	748	118	11.9
	L.V. Affected	779.0	23.2	28.2	91.2	9.3	202	205	796	748	114	11.7
	Change	-1.7	-0.8	-1.8	-2.9	-0.1	-7	-12	+9	0	-4	-0.2
	Sep. Control	778.9	22.4	30.3	92.7		195	219	793	746	117	
	Sep. Affected	777.8	23.7	32.6	88.9		206	235	780	742	113	
	Change	-1.1	+1.3	+2.3	-3.8		+11	+16	-13	-4	-4	
	R.V. Control	791.3	25.5	33.8	90.6		225	248	764	760	117	
	R.V. Affected	788.0	25.7	33.1	93.6		227	243	765	758	121	
	Change	-3.3	+0.2	-0.7	+3.0		+2	-5	+1	-2	+4	
Average of 6 controls, 4-288 hours post op- eratively	Serum	925.2	111.9	139.2								
	L.V. Control	783.3	24.0	30.9	85.2	9.8	219	231	775	748	111	126
	L.V. Affected	782.5	24.5	32.0	90.6	10.0	223	239	769	745	116	132
	Change	-0.8	+0.5	+1.1	+5.4	+0.2	+4	+8	-6	-3	+5	+6
	Sep. Control	782.6	23.3	31.8	90.6		213	232	776	746	116	
	Sep. Affected	779.9	23.8	32.7	88.5		217	238	771	744	113	
	Change	-2.7	+0.5	+0.9	-2.1		+4	+6	-5	-2	-3	
	R.V. Control	788.5	24.9	32.6	92.8		230	242	765	755	120	
	R.V. Affected	786.0	25.1	32.4	91.0		233	240	764	754	118	
	Change	-2.5	+0.2	-0.2	-1.8		+3	-2	-1	-1	-2	
	σ*	6.5	1.7	2.5	5.4		15	23	17	6	6	
	σ†	1.5	0.5	1.2	2.3		5	9	6	2	3	

\* Comparison with homologous regions of all 6 hearts.

† Two parts of same region of same heart compared.

in fact, in the same part of different hearts. As a result, the degree of ischemia produced by temporary occlusion would be expected to differ in the 3 divisions of the heart, and also in different hearts.

*Analyses of control hearts.* In table 1 are shown the results of 6 control experiments in which a mock occlusion of a coronary artery was performed and the tissue removed 4 to 288 hours later, 4 of the 6 being removed from 4 to 29



hours after operation. "Affected" signifies the region that would have been affected had the blood flow actually been interrupted by the ligature. One typical experiment is recorded completely; and in addition, the average for all the experiments is given together with the change between "affected" and "control" areas.

The average results fail to indicate any significant difference between "control" and "affected" portions, and it seems permissible to conclude that in the absence of actual occlusion the operative procedure employed did not materially affect any given region of the heart. No abnormal histological changes were evident in any of these hearts.

The data for these control experiments may, therefore, be examined to see what variations occur between different regions of the same heart or between the same regions of different hearts. As a measure of these variations, the standard deviations have been recorded in two different ways. First, there is given the standard deviation of individual values from the average value for all of the tissues from homologous regions of the 6 hearts—e.g., individual left ventricle values are compared with the average of all left ventricle values. Second, there is given the standard deviation of individual values from the average of the two values, "control" and "affected," for the same part of the same heart;—e.g., an individual value for the left ventricle "affected" is compared with the average of the left ventricle "affected" and "control" in the same heart.

As might have been anticipated, the correlation is much better between nearby regions of the same heart than between homologous regions of different hearts. Therefore, in the case of actual occlusions, there appears to be a real advantage in obtaining control samples from the same heart, and from each of the ventricles and the septum of that heart.

On the average, there is good correlation between the relative amount of extracellular tissue calculated from chloride,  $E_{Cl}$ , and that calculated from sodium,  $E_{Na}$ . However, the extracellular values estimated from the sodium data averaged 7 per cent greater than those calculated from the chloride figures, which suggests the presence of a small amount of intracellular sodium, perhaps 3 milliequivalents per kilogram of fibers. In spite of the considerable variation in the amount of extracellular fluid in different hearts, the water content of the fibers,  $(H_2O)_C$ , showed only moderate fluctuations.

The relative amount of extracellular tissue in the control hearts varied from 200 to 260 grams per kilogram of blood-free, fat-free tissue. Slightly higher values were found in the right ventricle than in the septum or left ventricle. This difference, although small, is consistent with the work of previous investigators who found higher chloride and water concentrations in the right ventricle than in the left (17) (18). The right ventricle thus appears to be a little more loosely constructed than the rest of the myocardium.

*Analyses of hearts removed 4 to 5 hours after temporary arterial occlusions.* In table 2 are recorded the quantitative chemical changes present in the hearts of 4 dogs, 4 or 5 hours after the termination of temporary occlusion of a coronary artery. The actual occlusions lasted 15 minutes in one case, and 45 minutes in the rest. The results in 2 typical cases and the average of all 4 are presented.

In general, there is an increase in the water, chloride, and sodium concentrations in the myocardium with no conclusive change in potassium or glycogen.

TABLE 2

*Analyses of hearts removed 4 to 5 hours following temporary arterial occlusions*

(H<sub>2</sub>O)<sub>c</sub>, (K)<sub>c</sub>, and (Gly)<sub>c</sub> calculated per kilogram of fibers.

Other data based on a kilogram of fat-free, blood-free tissue.

		ORIGINAL DATA					DERIVED DATA					
		H <sub>2</sub> O	Cl	Na	K	Gly-cogen	E <sub>Cl</sub>	E <sub>Na</sub>	C	(H <sub>2</sub> O) <sub>c</sub>	(K) <sub>c</sub>	(Gly) <sub>c</sub>
		grams	mEq.	mEq.	mEq.	grams	grams	grams	grams	grams	mEq.	grams
No. 2*, 45 minutes occlusion; 4.5 hours after occlusion	L.V. Control	780.4	19.8	24.7	87.8	6.5	195	190	808	750	107	8.1
	L.V. Affected	786.3	24.8	28.8	82.4	7.6	241	219	770	748	105	9.9
	Change	+5.9	+5.0	+4.1	-5.4	+1.1	+46	+29	-38	-2	-2	+1.8
	Sep. Control	777.9	20.8	24.5	86.0		204	188	804	748	105	
	Sep. Affected	787.0	23.6	28.9	89.2		230	220	775	752	114	
	Change	+9.1	+2.8	+4.4	+3.2		+26	+32	-29	+4	+9	
	R.V. Control	772.9	19.5	24.2	87.9		196	190	807	748	108	
	R.V. Affected	779.1	20.7	25.1	90.1		207	197	798	752	111	
	Change	+6.2	+1.2	+0.9	+2.2		+11	+7	-9	+4	+3	
No. 3, 45 minutes occlusion; 4 hours after occlusion	L.V. Control	781.3	27.0	32.4	88.8	6.3	247	243	755	739	116	8.3
	L.V. Affected	781.0	29.7	33.4	88.9	6.7	270	251	740	734	115	9.1
	Change	-0.3	+2.7	+1.0	+0.1	+0.4	+23	+8	-15	-5	-1	+0.8
	Sep. Control	779.5	25.9	31.4	89.4		237	236	764	738	116	
	Sep. Affected	806.2	46.2	59.8	76.2		412	438	575	712	130	
	Change	+26.7	+20.3	+28.4	-13.2		+175	+202	-189	-26	+14	
	R.V. Control	781.0	28.0	34.4	91.4		259	261	740	739	122	
	R.V. Affected	786.2	30.1	35.9	94.3		279	273	724	743	128	
	Change	+5.2	+2.1	+1.5	+2.9		+20	+12	-16	+4	+6	
Summary of 4 hearts removed 4-5 hours after occlusion	L.V. Control	785.5	23.8	29.3	88.2	6.6	214	218	785	752	111	8.4
	L.V. Affected	787.0	28.8	32.2	87.3	7.0	241	237	761	748	112	9.4
	Change	+1.5	+5.0	+2.9	-0.5	+0.4	+27	+19	-24	-4	+1	+1.0
	Sep. Control	783.5	23.2	29.0	91.5		210	214	789	747	115	
	Sep. Affected	793.8	34.0	38.4	83.2		260	281	736	733	121	
	Change	+10.3	+10.8	+9.4	-3.3		+50	+67	-53	-14	+6	
	R.V. Control	782.7	24.1	30.7	95.1		224	233	773	746	123	
	R.V. Affected	785.4	26.4	32.8	93.6		245	245	755	744	123	
	Change	+2.7	+2.3	+1.1	-1.5		+21	+12	-18	-2	0	

\* The dogs numbered 1, 2, 3, 4, 5 in this paper correspond with those numbered 71, 70, 69, 54, 55, respectively, in the paper (3) in which the electrocardiographic and morphological studies have already been reported in detail.

The failure to observe the fall in glycogen that others have found may be due to the relatively short duration of these occlusions. The different divisions of the heart evidence different degrees of change, as is anticipated from the variation

in the extent to which the affected regions were dependent on the occluded artery for their blood supply.

When these analytical data are interpreted histochemically, the changes appear to involve an average increase of 15 per cent in the proportion of extracellular tissue, the equivalent of a 3 per cent fall in the relative mass of fibers, without significant change in the water or potassium concentration in the muscle fibers.

These conclusions rest on the assumption that chloride and sodium did not enter the muscle fibers as a result of the temporary ischemia. Support for this assumption may be found in the fact that *a*, approximately the same change in extracellular fluid was found whether sodium or chloride was used as a measure of the extracellular fluid, and *b*, the concentration of water in the fibers appeared to remain constant when calculated on this same assumption. Therefore, either chloride and sodium remained outside the fibers, as assumed, or, which seems less likely, chloride, sodium, and water must have entered the fibers in just the proportions existing in the extracellular fluid.

With these reservations, one may conclude tentatively that 4 or 5 hours after a short period of ischemia, there appears to be a moderate extracellular edema without evident change in the cardiac fibers. It is of interest that no abnormal morphological changes were detected either grossly or microscopically in these hearts (3).

Possibly this increase in extracellular fluid may be ascribed to damage to the small blood vessels of the heart. Tennant *et al.* (7) concluded that the capillary walls were altered so as to permit exudation following a 2 hour temporary arterial occlusion. Bronson (8) observed a marked increase in lactic acid in the myocardium within 1 or 2 minutes after the interruption of coronary blood flow. Other workers (19) (20) have concluded that acid production in conjunction with the release of specific vasodilators, was responsible for hyperemia subsequent to a temporary arterial occlusion. In this connection, it may be pointed out that either 15 or 45 minute occlusions resulted in an edema that was present 4 hours after the insult, whereas only occlusions lasting longer than 20 minutes produced permanent histological lesions (3). Conceivably, the shorter occlusions injure only the blood vessels, whereas the longer occlusions may injure both vessels and muscle fibers.

*Analyses of hearts removed 24 hours or more after the termination of temporary arterial occlusions.* In 4 instances, following the temporary occlusions, 1 to 16 days were allowed to elapse before the removal of the heart. In 2 of these instances, the occlusions lasted 20 minutes and the animals were allowed to survive 24 and 26 hours. No material changes were observed in the chemical constituents of either of these hearts.<sup>3</sup> These negative results are consistent with the general observations of Blumgart *et al.* (3) that there are seldom any permanent histological changes found in the myocardium following arterial occlusions of 20 minutes or less. It would appear that although an increase in extracellular fluid occurs following occlusions lasting only 15 or 20 minutes, this increase is only transitory and subsides within 24 hours after the operation.

<sup>3</sup> A minute area of necrosis, not obvious on gross examination, was found by Dr. M. J. Schlesinger on microscopic examination in one section of one of these hearts.

Temporary occlusions lasting longer than 20 minutes usually result in permanent morphological changes in the myocardium, detectable both grossly and microscopically. Two such cases were examined chemically (table 3). The arterial occlusions lasted 45 minutes and the hearts were removed 14 and 16 days postoperatively. At this time, the affected region (left ventricle) in both hearts showed a marked increase in water, chloride, and sodium. The potassium concentration was definitely decreased in the affected portion of the one heart for which values were obtained. Calcium was increased in both cases, particularly in one case in which a 70-fold rise occurred. These changes in water and electrolytes are similar to those observed by Wilkins and Cullen (21) in the hearts of human patients dying of cardiac failure.

The derived histochemical data for these two hearts indicate a relative increase in the extracellular tissue of the affected portions compared to the control

TABLE 3

*Analyses of hearts removed 2 weeks or more after the termination of temporary arterial occlusions*

(H<sub>2</sub>O)<sub>c</sub>, (K)<sub>c</sub> calculated per kilogram of fibers.

Other data based on a kilogram of fat-free, blood-free tissue.

			ORIGINAL DATA					DERIVED DATA				
			H <sub>2</sub> O	Cl	Na	K	Ca	ECI	ENa	C	(H <sub>2</sub> O) <sub>c</sub>	(K) <sub>c</sub>
			grams	mEq.	mEq.	mEq.	mEq.	grams	grams	grams	grams	mEq.
No. 4; 45 minutes occlusion; 14 days after occlusion	L.V. Control		797	32.4	36.4	82.4	2.0	296	268	718	750	113
	L.V. Affected		807	37.0	42.2	75.5	3.0	337	309	677	753	111
	Change		+10	+4.6	+5.8	-6.9	+1.0	+41	+41	-41	+3	-2
No. 5; 45 minutes occlusion; 16 days after occlusion	L.V. Control		796.0	39.9	44.7		2.1	360	335	652	728	
	L.V. Affected		805.5	46.5	56.1		154	422	420	579	728	
	Change		+9.5	+6.6	+11.4		+152	+62	+85	-73	0	

regions. Even the control regions of both hearts contained more extracellular tissue than normal. This change in the control portions might be correlated with the extra burden placed on the rest of the myocardium by injury to the region directly affected by the ischemia.

There was no difference in water concentration in the fibers of the control and of the affected regions, although in the second heart the water concentration in both control and affected regions was rather low. The possibility of increases in the amount of collagen fibers, especially in the affected regions, would have to be considered in deciding the exact water concentration in the fibers, since an increase in collagen, if not evaluated, would make the fiber water concentration appear too low.

The potassium concentration in the fibers was within normal limits. The calcium concentration was increased in the affected regions in both hearts. In the second heart (dog 5), the affected area contained calcium equivalent to 7.5 grams of Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> per kgm. of cardiac muscle. This extra amount of

solids was subtracted from the other solids before calculating the water content of the fibers.

In both hearts calcium deposits were observed in sections used for morphological study.

The histochemical changes in the heart 2 weeks after a 45 minute coronary occlusion, therefore, appear to consist in part of a relative increase in the amount of extracellular tissue (or rather, a relative decrease in intracellular tissue) without definite changes in the composition of the remaining fibers. It is of interest that definite morphological changes were found in these hearts. The histological findings were those of healing myocardial necrosis (3).

*Fibrillation and partial thrombosis.* Two additional hearts were studied chemically. In one heart, the ventricles went into fibrillation  $4\frac{1}{2}$  minutes after the beginning of the coronary occlusion and then into complete standstill within a few minutes. The electrolyte composition of this heart was within normal limits, and no histological changes could be detected.

In another case, after an operation planned as a control experiment, a thrombus was found which partially occluded the left circumflex coronary artery. This heart, which was removed 24 hours after the operation, showed chemical changes indicating the presence of an increased amount of extracellular fluid.

#### SUMMARY

1. Dog hearts were examined chemically at various times following the termination of temporary occlusions of a major coronary artery. Characteristic increases in the water, chloride, and sodium content of the myocardium were observed. In general, there was little change in the potassium concentration.

2. These changes have been interpreted histochemically as denoting an increase in the proportion of extracellular fluid without demonstrable change in the muscle fibers themselves.

3. Occlusions lasting 20 minutes or less produced an increase in extracellular fluid which persisted 4 hours but not 24 hours. Occlusions lasting 45 minutes produced a relative increase in the amount of extracellular tissue present both 4 hours and 2 weeks after the occlusion.

We are deeply indebted to Dr. H. L. Blumgart and Dr. M. J. Schlesinger for their participation in this investigation, and for their guidance in the preparation of the manuscript.

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# SOME SPECIAL INSTANCES OF PREDOMINANT CHEMICAL STIMULATION OF THE EXPIRATORY HALF-CENTER

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The first change in breathing during pneumothorax is a powerful and more or less protracted inspiratory contraction which in due time is interrupted by a relatively weak expiratory contraction. From this point on both inspiratory and expiratory contractions grow in strength. With the first inspiratory contraction almost at maximum the expiratory contractions gain the more rapidly. As the intensity of inspiratory and expiratory components approximate each other, inspirations are interrupted at an earlier moment and the frequency of breathing is thereby accelerated. But later when the expiratory component gains dominance over the inspiratory act the expiratory act becomes disproportionately prolonged thus holding the inspiratory act in abeyance and retarding the frequency of breathing. The disproportionate increase of expiratory activity is apparently the critical factor involved in the changing events of breathing during pneumothorax. It is, therefore, significant that oxygen lack should play such an important rôle in the augmentation of expiratory activity (Gesell and Moyer, 1942). (See also figs. 2A and B of the present experiments.) The nature of the chemical stimulation associated with this phenomenon seemed worthy of further consideration.

**RESULTS.** *The nature and site of chemical stimulation of the expiratory half-center.* In figure 2A the dog was breathing room air when pneumothorax was established. Due to the relatively low sensitivity of the respiratory reflexes the progressive changes in breathing were small. Yet with the aid of a straight edge placed along the upper limits of the expiratory tracings during pneumothorax the increased expiratory activity becomes apparent. A few minutes later, with the dog now breathing an oxygen-poor mixture, the course of breathing during pneumothorax is greatly modified. The expiratory component grows more rapidly and breathing is more strikingly retarded. These effects of low oxygen occur with the greatest regularity and consequently are of interest in a comparison with the effects of other well-known forms of respiratory stimulation during pneumothorax.

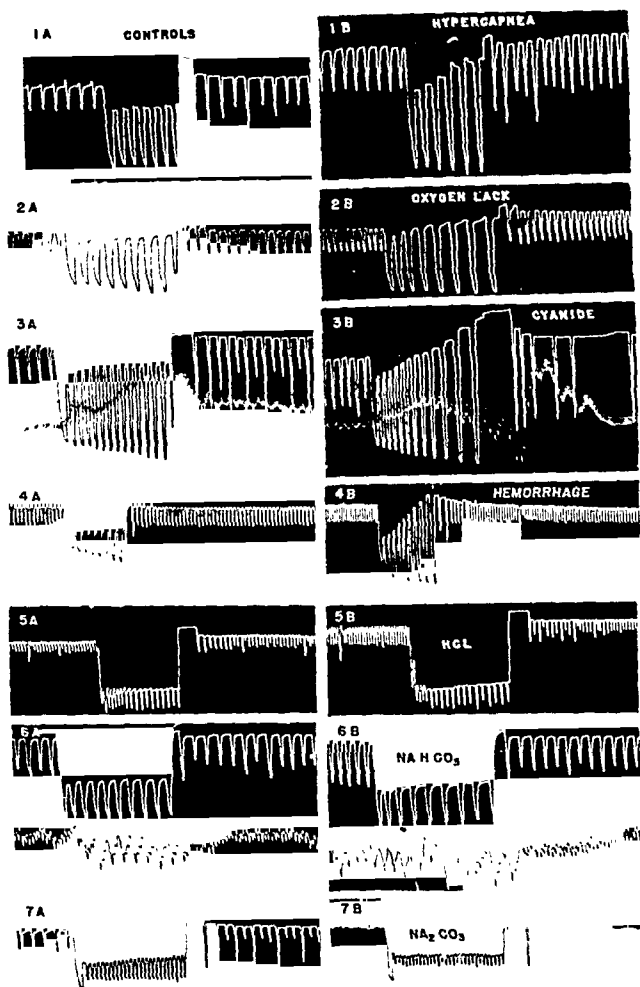
The first and foremost of these forms of stimulation is hypercapnia which exerts the same profound effect upon the respiratory response to pneumothorax as oxygen lack. Expiratory activity and retardation of breathing are markedly increased (see figs. 1A and 1B).

During cyanide poisoning, if the injection is sufficiently large, the intensification of expiratory activity and retardation of breathing may be exceptionally striking (see figs. 3A and 3B).

Hemorrhage also exerts a pronounced influence on the response of breathing to pneumothorax (see figs. 4A and 4B).

In figures 5A and 5B the effects of intravenous injection of hydrochloric acid are obvious. As the expiratory circumference diminishes frequency of breathing is retarded.

Last of the respiratory stimulants which we studied is sodium bicarbonate (see figs. 6A and 6B). It is to be noted first of all that bicarbonate actually



Figs. 1 to 7. Examples of expiratory augmenting action of asphyxia superimposed upon simple open pneumothorax. Breathing is recorded by the torso band method (inspiration is indicated by a down stroke). The left column of figures shows the changes of breathing produced by simple pneumothorax. They serve as controls for the right hand figures which show the effects of added asphyxias.

increases the breathing. (See the initial 5 breaths of fig. 6B and compare with those before pneumothorax in fig. 6A. Also compare the retarded pulse in fig. 6B with that of 6A.) Secondly, it is to be noted that the response to pneumothorax has been altered by sodium bicarbonate in a manner similar to that of hypercapnia, oxygen lack, cyanide, hemorrhage and hydrochloric acid. Intensified expiratory constriction is accompanied by greater retardation of breathing.



Only following administration of sodium carbonate does the frequency of breathing fail to be retarded during pneumothorax. Almost complete elimination of respiratory movements before the onset of pneumothorax is indicative of the high degree of hypocapnia produced by the injection. The well sustained accelerated frequency of breathing during pneumothorax is therefore thought to be significant.

Granting that the degree of retardation of breathing under our experimental conditions is an expression of the intensity of chemical stimulation of the expiratory half-center these results give promise of further information on the details of the chemical control of breathing, essential to the explanation of our present findings. Be it further conceded that cH plays a major rôle in the adjustment of breathing, the variety of forms of stimulation which we have studied offers singular opportunities to re-analyse the possible site of action of the hydrogen ion for, so far as we know, selective chemical stimulation of the expiratory half-center has never been investigated. Such a reconsideration from a new angle is all the more timely in the light of recent criticisms of the cH theory of respiratory control by Nielsen (1936), Krogh (1941) and Schmidt (1938, 1941)—containing many contradictions of a vital nature.

Surely there can be no doubt of a greater cH of the tissues and of the blood when combining pneumothorax with hypercapnia or intravenous injection of hydrochloric acid as compared with pneumothorax under otherwise normal conditions. There is more free acid present and the means of immediate elimination have been abolished. The increased stimulus of the expiratory half-center could, therefore, have been the increased cH of the blood or the increased cH of the nerve cells of the respiratory arc. Neither can there be any doubt of the state of the acid-base equilibrium existing during pneumothorax plus cyanide injection. Greater acid formation than normal added to the handicap of zero pulmonary ventilation must turn both the tissues and blood more acid than normal. The situation with hemorrhage is the same. A gradual accumulation of acid resulting from a subnormal volume flow of blood before pneumothorax plus a smaller sum-total of oxygen-saturated blood at the time of pneumothorax must assure a higher degree of tissue asphyxia. Both blood and tissues must turn more acid.

In the case of pneumothorax plus oxygen lack the universal increase of cH in tissue cells and blood is not so certain for cH determinations were not made. The possibility must be admitted that the blood may not have turned more acid than during pneumothorax following the breathing of room air due to the counteracting effects of a preceding reduction of oxyhemoglobin. On the other hand, it seems highly probable that the cells themselves did turn more acid than normal due to the rapid formation of anaerobic acid within them. This particular situation, therefore, might conceivably favor tissue cH as the possible site of action of respiratory adjustment as against blood cH.

The acid-base disturbances produced by sodium bicarbonate are much clearer in this respect. On theoretical grounds supported by experimental observations it may be safely inferred that intravenous injection of sodium bicarbonate is

capable of turning the interior of the cells more acid while at the same time lowering the hydrogen ion concentration of the blood. Direct experiment shows that cerebrospinal fluid, which is separated by a barrier membrane that blocks the free entrance of metallic ions from the blood, becomes more acid though the blood turns sharply alkaline (Gesell and Hertzman, 1926). Similar acid changes undoubtedly occur within the cells of the expiratory half-center when bicarbonate is injected. The greater inhibition of the heart during pneumothorax plus sodium bicarbonate (fig. 6B) is rather strong evidence that pertinent stations in the cardio-inhibitory reflex arcs turned more acid than during simple pneumothorax. Therefore, if it be either the cH of the blood or the cH of the nerve cells which determines expiratory activity the decision must be in favor of the latter.

The absence of expiratory excitation during pneumothorax plus the injection of sodium carbonate could accordingly be attributed to the alkaline state of the expiratory half-center caused by excessive diffusion of carbon dioxide out of the center into the blood.

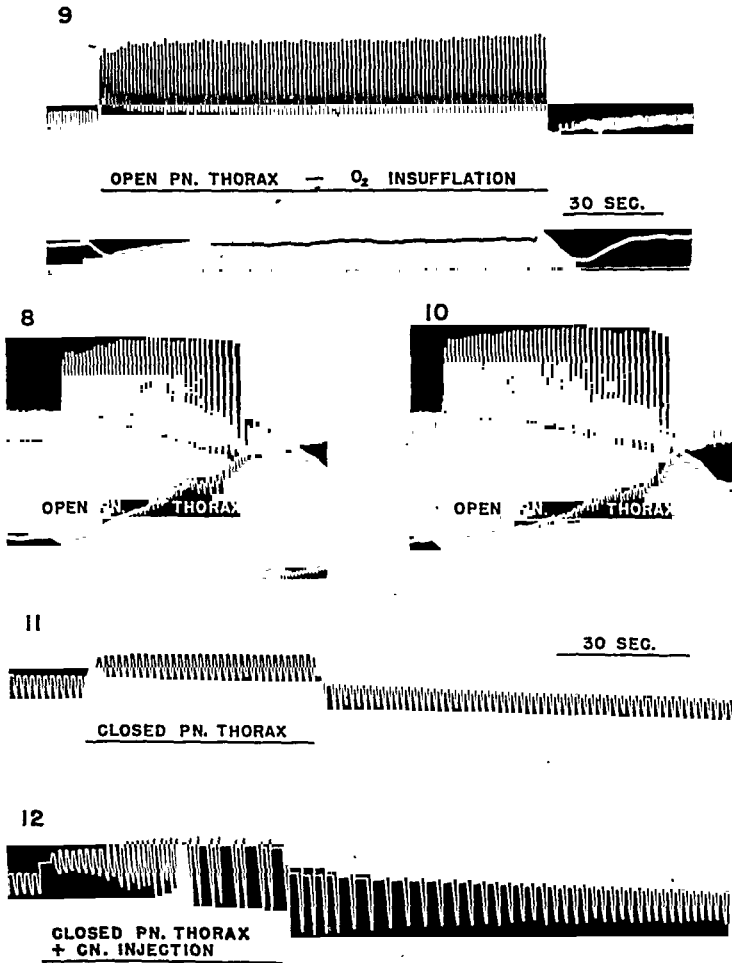
The cH gradient from cell to environment has also been considered as a factor determining nerve cell activity (see Andrus and Carter, 1924, and Andrus, 1924). A comparison of sodium bicarbonate and carbonate effects is pertinent on that score. Since both salts turn the blood more alkaline with respect to the cell and thus increase the outward gradient of cH they might be expected to have a similar action, yet their effects on breathing are diametrically opposite.

Another pertinent comparison is found in the effects of sodium bicarbonate and those of oxygen administration after a period of oxygen lack because of the probability that oxygen also produces opposite changes in the cH of the blood and tissues. However, in the case of oxygen it is the *blood* that turns acid and the tissues alkaline. Now if it is the cH of the blood that determines the degree of stimulation, breathing should increase. If, on the other hand, it is the cH of the tissues that matters, breathing should diminish as it does.

It is, therefore, clear that blood cH is at least not the determining factor in respiratory control, that an increase of free hydrogen in the blood ( $O_2$  administration) can be incapable of increasing ventilation and that a decrease of free hydrogen ions in the blood (sodium bicarbonate administration) can be incapable of diminishing breathing. As we see it the explanation of chemical control of breathing rests primarily on cellular cH and *all* related factors affecting it (rate of acid formation, amount of acid in the cells and blood, the forces leading to movement of acid in either direction, the amount of buffers present, semipermeability of membranes, etc.). We believe that the failure of Nielsen, Krogh and Schmidt to recognize these facts in their entirety and the failure to appreciate that cellular and blood cH are not synonymous have led them into their present position.

Our first conclusion with regard to the origin of the increased expiratory activity during pneumothorax is that increased nerve cell acidity plays an important rôle. This conclusion receives additional support in figure 9 (please note that upstroke indicates inspiration in figs. 8, 9 and 10) in which asphyxia,

instead of being intensified during pneumothorax, is prevented by a gentle oxygen insufflation. Breathing then continues for an indefinite duration of time without the development of excessive expiratory activity such as seen in the control figures 8 and 10 where asphyxia is allowed. The steeply rising



Figs. 8, 9 and 10. The absence of augmentation of expiratory activity during open pneumothorax when asphyxia is prevented by gentle insufflation of the lungs with oxygen (fig. 9). In the control observations figures 8 and 10 where asphyxia is allowed excessive expiratory activity develops (inspiration is indicated by upstroke).

Figs. 11 and 12. The absence of development of excessive expiratory activity during closed pneumothorax when the animal's own breathing prevents the appearance of asphyxia (fig. 11). Addition of tissue asphyxia by the injection of cyanide initiates excessive expiratory activity and retardation of breathing (fig. 12). (Inspiration in fig. 11 and 12 is indicated by downstroke.)

blood pressure and the increasing cardio-inhibition show the intensity of asphyxia when insufflation is omitted.

*The reflex factor of dominant expiratory activity.* It is hardly probable that the slight pulmonary inflation produced by oxygen insufflation plays a signifi-

cant rôle in preventing excitation of the expiratory half-center. This follows from the respiratory response to closed pneumothorax when the chest is sealed after the lungs have collapsed during the first stages of open pneumothorax (figs. 11 and 12). Under these conditions both partial inflation and ventilation of the lungs obtain and breathing often reaches a steady state similar to that in figure 9 (see fig. 11). But if asphyxia is added to this situation by injection of sodium cyanide, the picture changes (fig. 12). Expiratory activity builds up to supernormal levels and retarded breathing comes on. Under ordinary conditions simple cyanide asphyxia produces a very rapid rate of breathing. What then is the modifying factor which creates this characteristic retardation of breathing?

Figures 13, 14 and 15 show that complete abolition of vagal reflexes by cold block does not prevent a progressive development of expiratory activity similar in general aspects to that already noted. If there is a proprioceptive factor involved it must be of extra vagal origin. Whatever this factor may be it fails to reveal its effects during vagal block if asphyxia is prevented by oxygen insufflation (fig. 15). A slight increase of expiratory activity will be noted which is commensurate, however, with the small degree of asphyxia (note the slowly rising blood pressure). It is interesting to note the effects of oxygen insufflation during vagal block in the absence of pneumothorax. There was an invariable reduction of expiratory activity, augmentation of rhythm and reduction of tidal air (figs. 14 and 15).

Head showed many years ago that blocking of the vagus nerves may produce a long series of slow, powerful and prolonged inspirations in which the torso is markedly stretched and the total ventilation is impaired. The causes of such breathing have been considered by Gesell and Moyer (1942) an example of which is illustrated in figure 16. The slowly rising blood pressure may be regarded as a reasonably safe index of a slowly mounting asphyxia and as this asphyxia increases expiratory activity grows stronger and longer until rhythmic breathing finally stops in a protracted and strong expiratory contraction. Deblocking of the vagi leads to recovery. Now this situation is different from all those mentioned above in that neither pneumothorax nor collapse of the lungs was essential for the development of excessive expiratory activity and yet there are two important points of agreement with pneumothorax which give it value in the present consideration. These are progressive asphyxia plus a well sustained over-distention of the torso capable of setting up new proprioceptive drives.

By the process of exclusion certain factors (pneumothorax, lung volume, lung immobility and vagal reflex activity) seem to be eliminated as important in the development of excessive expiratory activity and its associated retardation of breathing. Asphyxia and increased torso volume remain as the only invariable accompaniments of expiratory activity. Though simple open pneumothorax invariably gives rise to excessive expiratory activity its effects seem to resolve themselves into the factors of asphyxia plus increased torso volume. It, therefore, seems reasonable that any circumstances giving rise to a combina-

tion of these two conditions may be responsible for dominant expiratory activity. Precisely how these two conditions might function however is not known. Whether both are absolutely essential, whether one is more important than the other cannot be definitely concluded. It is conceivable that acidity

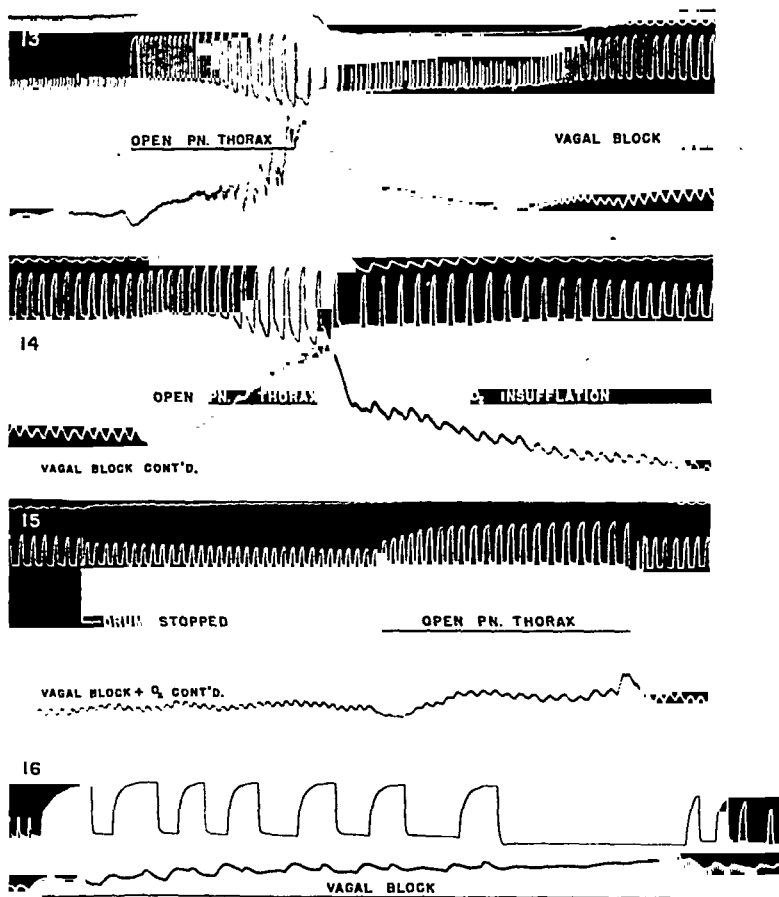


Fig. 13. Typical development of excessive expiratory activity during open pneumothorax with vagi functioning. Subsequent vagal block shows a deepening and slowing of breathing.

Fig. 14. Development of excessive expiratory activity during open pneumothorax and double vagal block. Oxygen insufflation subsequent to open pneumothorax decreases expiratory activity, increases frequency of breathing and diminishes tidal air.

Fig. 15. Oxygen insufflation during double vagal block diminishes the expiratory response to open pneumothorax.

Fig. 16. Simple vagal block which produces powerful, infrequent inspiration, plus asphyxia is accompanied by a progressive increasing dominance of expiratory activity. (Upstroke indicates inspiration in figs. 13, 14, 15 and 16.)

works directly upon the centers. All things considered, it appears more probable that the effects of these two factors are in some way interrelated. It is suggested that an increased torso volume initiates an expiratory reflex drive which in turn is intensified by asphyxia.

## SUMMARY AND CONCLUSIONS

Special instances of predominant chemical stimulation of the expiratory half-center occurring during pneumothorax and simple vagal block were analysed in relation to the nature and site of chemical stimulation and in respect to a possible interaction of the chemical and proprioceptive stimulation.

Simple open pneumothorax increased the activity of the expiratory half-center and gave rise to a progressive dominance of that half-center over the inspiratory half-center. During such dominance the inspiratory half-center is held in increasing abeyance. Frequency of breathing diminishes in proportion to the increasing duration and strength of the expiratory act.

Asphyxia is definitely a most important contributing factor in the expiratory domination of breathing during pneumothorax for the addition of well-recognized forms of respiratory stimulation accelerate and increase the growth of expiratory activity. This was found to be true for hypercapnia, oxygen lack, cyanide, hemorrhage, hydrochloric acid and sodium bicarbonate.

Previous double vagal block did not prevent the occurrence of excessive expiratory stimulation during pneumothorax. If proprioceptive reflexes are essential they must consequently be extra vagal in origin.

When simple vagal block (chest intact) produced slow, powerful and prolonged inspiratory contractions and, therefore, deficient pulmonary ventilation expiratory activity also attained dominance of the respiratory act as asphyxia mounted. It is, therefore, concluded that neither pneumothorax nor vagal reflexes are essential to such dominance.

The two outstanding factors seem to be an increasing asphyxia and an associated modification of extra vagal proprioceptive drive possibly arising in the over distended torso.

It is tentatively suggested that asphyxia in some way increases the effectiveness of the incoming respiratory nerve impulses which under the conditions of our experiments have a high expiratory excitatory value.

The dominant chemical stimulation of the expiratory half-center allowed singular opportunities for a reanalysis of the nature and site of chemical stimulation. The evidence gathered by this new method of approach points to the importance of the rôle of the hydrogen ion and to an intracellular site of action.

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# THE EFFECT OF SULFANILAMIDE ON THE ABILITY OF RABBITS AND DOGS TO WITHSTAND HIGH ALTITUDES

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The use of sulfanilamide as a chemotherapeutic agent prompted study of the effects of this drug on resistance to altitude. Flying personnel undergoing therapy with this drug are grounded, so that while the rationale of this precaution was not questioned, the effects of the drug on the functions of the organism were thought to deserve study. In addition, the advent of the air-ambulance rendered it advisable to determine, if possible, whether special precautions should be taken in such flights, if the patients have been receiving sulfanilamide.

**METHODS.** The animals were examined in a small decompression chamber, which was two feet in diameter and four feet in length. It was fitted with three windows and with numerous outlets. The outlets consisted of semi-couplings with removable plugs. They were utilized to permit sampling of arterial or venous blood or to obtain samples of alveolar air at various stages of decompression. They could also be used to record, outside the chamber, blood pressure, respiration, electrocardiograms, etc., or to inject into the animals any desired drug. An electrically-operated (Nash Hytor) vacuum pump permitted decompression of the chamber up to a rate comparable to ascents at 15,000 ft. per minute. Recompression could be accomplished in a few seconds. Coarse and fine inlet and outlet valves allowed accurate control. The chamber was made for the Department of Medical Research in the University of Toronto, by the Dominion Bridge Company.

When dogs were used, they were lightly anesthetized with nembutal, but no general anesthetic was given to the rabbits. In these animals the vessels were cannulated under local anesthetic with novocaine. When blood samples were taken, blood coagulation was prevented by heparin given intravenously.

*Arterial blood samples* were taken in rabbits by cannulating the proximal and distal ends of a common carotid artery. In dogs the femoral artery was employed. Blood from the proximal end of the vessel passed outside the chamber through a rubber stopper in one of the semi-couplings in one branch of a glass loop with a side-tube; in the other branch of this loop it returned through the same stopper to the distal end of the vessel. (See fig. 1A.) A continuation of the side-tube extended to a test-tube, where it ended beneath paraffin oil. This test-tube was connected with the interior of the chamber through another semi-coupling, so that the pressure within the tube could be rendered identical with that in the chamber. The side-tube was normally kept clamped; release of this clamp allowed the animal to bleed into the test-tube as the result of its own blood pressure. When adequate blood was obtained, the side-tube and the tube connecting the test-tube with the chamber could both be clamped. A tube

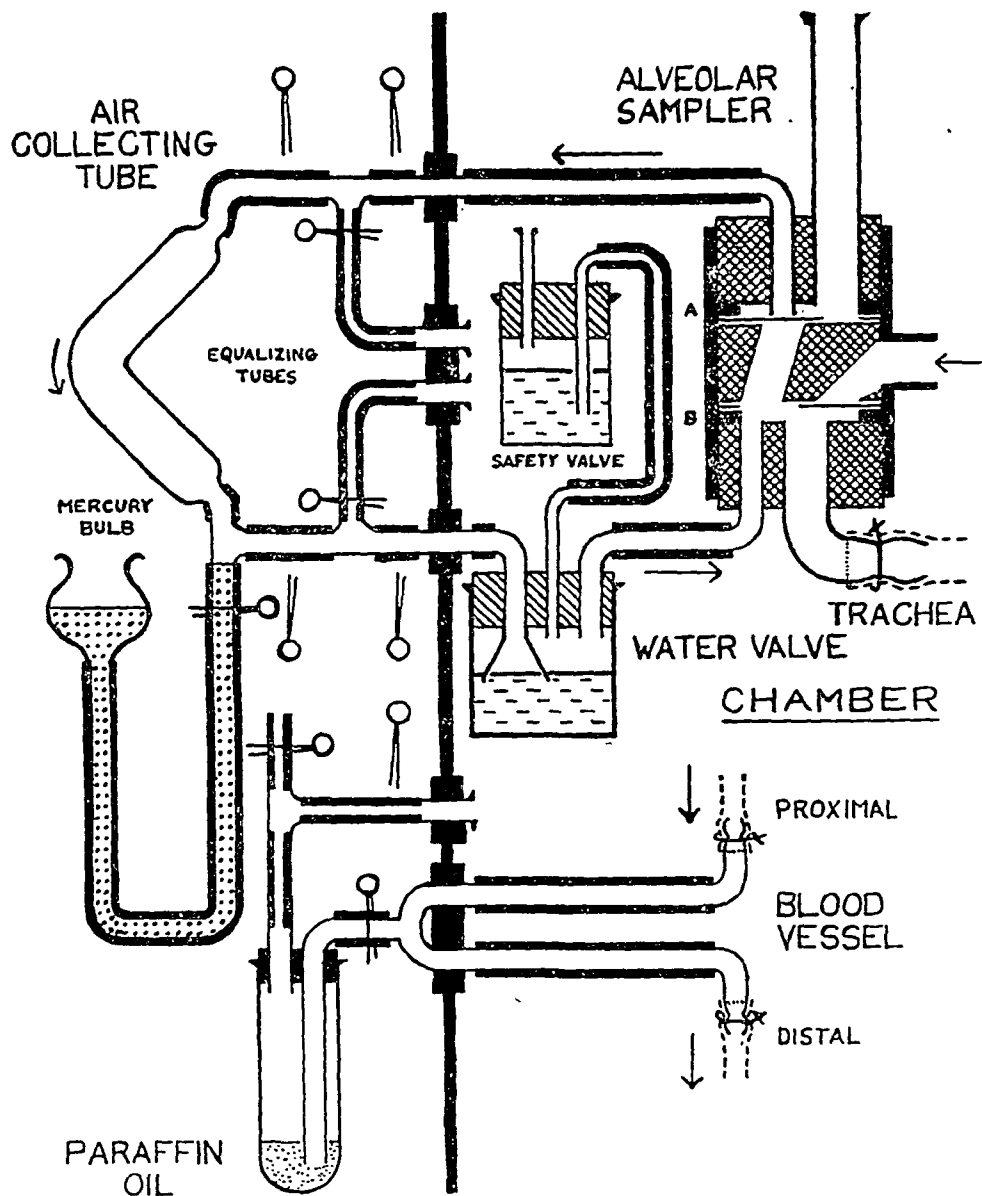


Fig. 1 A. Apparatus used for sampling alveolar air and blood from an animal while latter is decompressed to any simulated altitude. Parts to left of diagram are outside and parts to right are inside the decompression chamber, connections being made through semi-couplings in the chamber wall.

#### AIR STORAGE

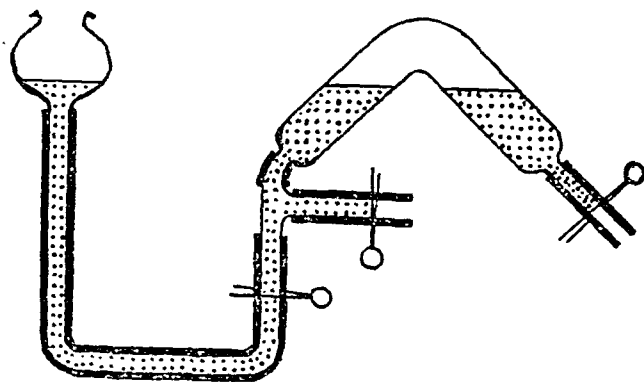


Fig. 1 B. Details of arrangement for storage of alveolar air.



connecting the test-tube with the outside air could then be opened and the tube be removed and replaced by a fresh tube. Before each sampling some blood was drawn and discarded to remove the blood stagnant in the side-tube (0.5 to 1.0 ml.). Precautions were taken to reduce heat loss from the exposed loops.

*Venous blood samples* were taken in a similar manner. The jugular vein was employed.

*The blood samples* so obtained could be handled by any of the accepted methods. The samples taken in the rabbits were 2 ml. in volume and the whole blood was analyzed for total  $\text{CO}_2$  and  $\text{O}_2$  content by the method of Van Slyke and Neill (1). In the dog experiments approximately 10 ml. were taken under paraffin oil into a tube with a constricted neck, and in most cases each sample was immediately centrifuged in a large cup filled with water at 38–39°C. Plasma was drawn off for  $\text{CO}_2$  analysis while the blood was maintained at approximately this temperature. In some cases whole blood was used, and in this case was analyzed for  $\text{CO}_2$  and  $\text{O}_2$ . Measurements were also made of the pH of either the plasma or of whole blood by the Beckman pH meter, utilizing a Beckman enclosed anaerobic glass electrode. The measurements were made when the sample was approximately at body temperature. Where corrections had to be made for the effect of temperature, an arbitrary correction of  $-0.005$  pH per degree C fall in temperature was used. Hastings and Sendroy's phosphate buffer mixtures were used as standards.

Arterial blood  $\text{CO}_2$  tensions were determined from the total plasma  $\text{CO}_2$  and the plasma pH by means of the nomogram of Van Slyke and Sendroy (1, vol. II, fig. 41). In some experiments, where  $\text{CO}_2$  content and pH determinations were made on whole blood, the plasma  $\text{CO}_2$  content was calculated from the line chart of Van Slyke and Sendroy (1, vol. II, fig. 39).

*Alveolar air samples* were taken by an automatic sampler utilizing the principle described by Mackay (2). In brief, each inspiration draws into an air-collecting tube 1 to 2 ml. of air from the end of the previous expiration. Inspiratory and expiratory valves are placed as close as possible to a short, low tracheal cannula and the air is drawn from a side-circuit. This brief description of the principle may be clarified by reference to figure 1A. The automatic sampler was made from a short length of iron pipe with a hole drilled in one side, three rubber stoppers with their sides ground perpendicularly and each bored with two holes, two rubber washers (3 mm. thick) with valve flaps of very thin rubber sheeting affixed to them, and four lengths of glass tubing. The dead space between the tracheal cannula and expiratory valve was 4.5 ml. At each inspiration 2 ml. of the last expiration beyond this valve were drawn off into the air-collecting tube or tubes. These tubes completed the alveolar circuit outside the chamber and were connected to the parts inside through the semi-couplings. The air-collecting tubes of 25 ml. capacity were allowed to fill for several minutes, after which they were closed by strong spring clips. The contained air was brought to or above atmospheric pressure, by means of a mercury levelling bulb, before they were disconnected from the system. Since the collecting tubes were outside the chamber they could be changed at will. These collecting tubes

were curved, so that they could be sealed with mercury at both ends (see fig. 1B) until analysis of the contained alveolar air was convenient. All outside rubber to glass connections were made of pure heavy gum rubber, if they had to be made or broken at altitude. For other outside connections pressure tubing was adequate if it was cemented to the glass.

Alveolar air analyses were carried out with samples of approximately 1.5 ml. volume in the Van Slyke-Neill apparatus, employing a modification of the Van Slyke-Neill method for estimation of  $\text{CO}_2$ ,  $\text{O}_2$ , and  $\text{N}_2$  (1).

**RESULTS.** *The absolute altitude tolerance* of many rabbits was determined. The animals were tested by exposure to a lowering of pressure comparable to an ascent at 1000 ft. per minute. The limit of tolerance was marked usually by the occurrence of one prolonged or several repeated gasps, occasionally by simple cessation of breathing. At such times the pupils were widely dilated. If the pressure in the chamber was rapidly restored (commonly to ground level) within 20 to 30 seconds, usually the rabbits could be resuscitated and sometimes they recovered spontaneously. Complete apparent recovery of the animal was the rule within five minutes. It was possible, therefore, to test rabbits when under sulfanilamide treatment and to compare the results obtained in control runs on the same animals before or after recovery from the treatment. Such experiments were carried out on 8 rabbits with 18 observations when under treatment, and with 21 control runs. The rabbits received sulfanilamide by stomach tube before the altitude test, and were tested under conditions such that the concentrations of sulfanilamide in their bloods ranged from 5 to 90 mgm. per 100 ml. When under treatment the average altitude tolerance was 40,700 ft., while in control runs it was only 36,100 ft. Nineteen other control tests were made in 12 other rabbits and indicated an average tolerance of 36,600 ft. Armstrong and Heim (3) have reported a figure for rabbits of 37,235 ft. After sulfanilamide 72 per cent of the tested animals reached 40,000 ft. or over, while this was the case in only 10 per cent of all controls. Great haste in restoring the pressure at the critical point seemed less essential after the drug, as gasping tended to be more prolonged. Most of the sulfanilamide-treated animals recovered spontaneously and none died, while in the controls many had to be resuscitated and six died.

There was therefore undoubtedly an increased tolerance to altitude in the treated animals. Since abdominal distention seriously affects respiration at high altitude (4) it seemed possible that any increased tolerance might be dependent on a lowered appetite, if the animals had received sulfanilamide for one or more days. Another series of control tests (25) was therefore run on 17 other rabbits after they had been deprived of food (but not water) for 24 hours. The average height reached was similar to that of normal rabbits, namely, 37,400 ft. The animals were taken to their absolute limit, since in 24 per cent resuscitation was not achieved.

Tests were also made in rabbits receiving smaller doses of sulfanilamide in the therapeutic range. Twenty-four tests were made on 14 animals, to which a single dose of 50 mgm. per kilo had been given 30 minutes before the test.

Such a dose gave a blood concentration of 3 to 7 mgm. per 100 ml. The average height reached was 39,800 ft., when control runs averaged 37,500 ft. Distribution graphs in figure 2 indicate the data on these and the other groups.

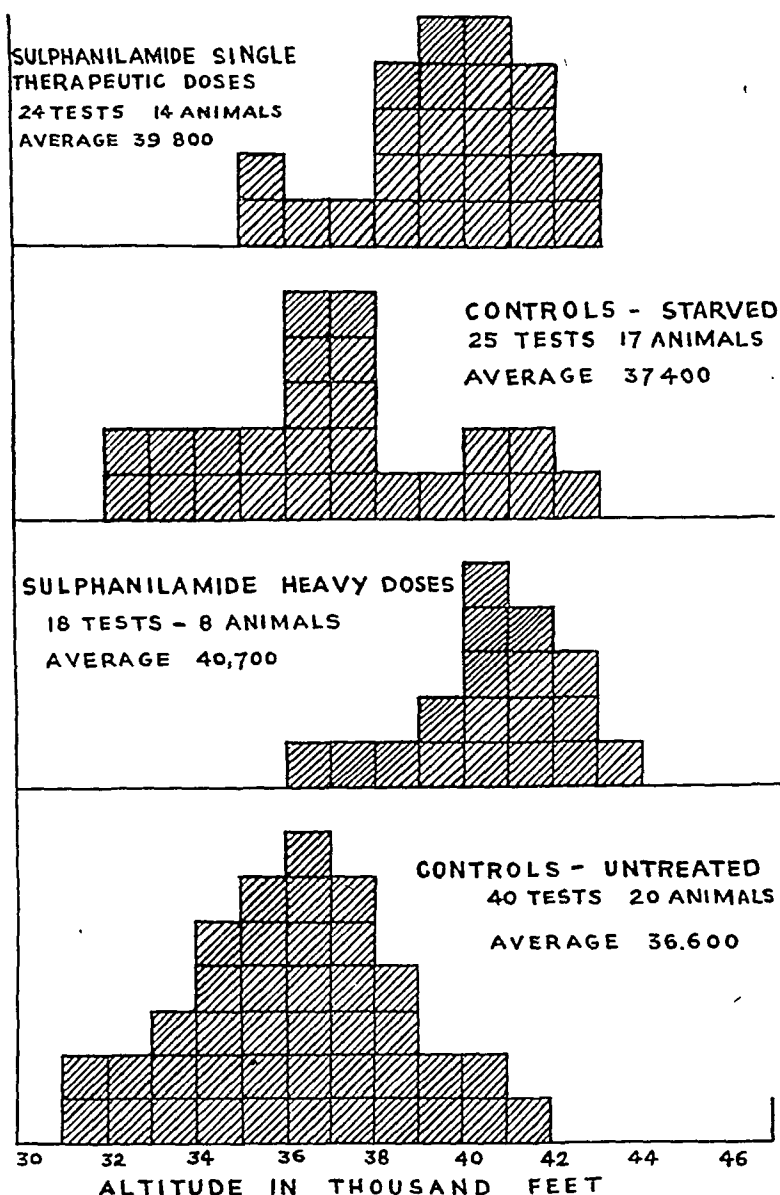


Fig. 2. Distribution graphs showing results obtained on decompressing sulfanilamide-treated and control rabbits to their absolute limit of tolerance at a rate corresponding to ascent at 1,000 ft. per minute.

*The levels of oxygen and carbon dioxide in the arterial blood of rabbits during decompression* were determined in many experiments. The animals were no doubt somewhat affected by the operative procedure in spite of the local anesthetic used. Thus in one control animal kept at ground level for a time similar

in duration to that of the experiments, the blood oxygen rose from an initial 19 to 20 volumes per cent and the carbon dioxide fell from an initial 41 to 34 volumes per cent. Such changes are unlikely to have interfered with the comparisons of the results obtained with and without sulfanilamide. In six control rabbits the initial average oxygen and carbon dioxide levels were respectively 15.7

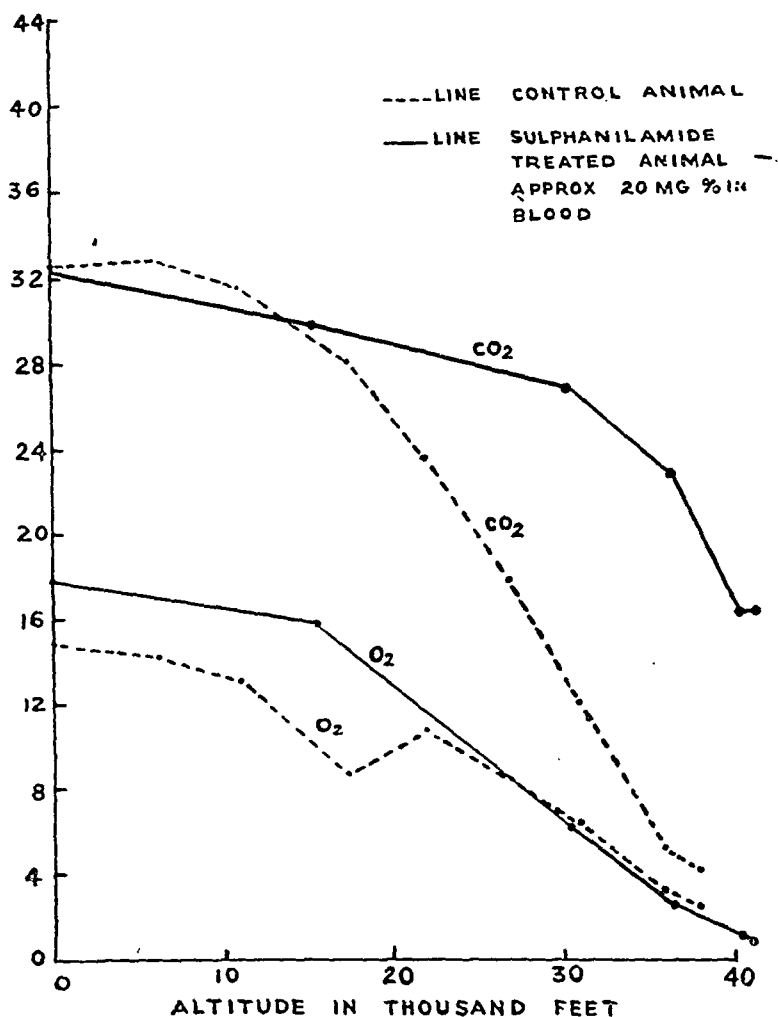


Fig. 3. Changes in blood CO<sub>2</sub> and O<sub>2</sub> content (in volumes per cent) of representative sulfanilamide-treated and control rabbits during decompression at a rate corresponding to ascent at 1,000 ft. per minute.

and 35.6 volumes per cent. Evidence of a fall in blood oxygen content appeared above 10,000 to 12,000 ft. and was accompanied by a fall in blood carbon dioxide, due to hyperventilation. At the limit of tolerance (38,400 ft.) the oxygen and carbon dioxide levels averaged 1.9 and 7.9 volumes per cent. In nine animals heavily dosed with sulfanilamide the average blood oxygen and carbon dioxide were 16.7 and 30.2 volumes per cent before decompression. The carbon dioxide values were therefore subnormal. During decompression the changes in oxygen

content resembled those of the controls, but the reduction in carbon dioxide was much slower. At the average limit of tolerance of 41,100 ft. the carbon dioxide of the blood averaged 13.7 volumes per cent. The fall in carbon dioxide was therefore much less than in the controls and the final values were higher, in spite of the initial low levels. The effects obtained in two animals are indicated in figure 3. The actual values obtained are given in table 1.

Three similar experiments were carried out on rabbits which received only 20 mgm. of sulfanilamide per kilo. All three animals started with a high arterial carbon dioxide level (averaging 44 vol. per cent) and attained very high altitudes (averaging 43,800 ft.), where the concentrations of carbon dioxide in the blood

TABLE 1  
*Blood O<sub>2</sub> and CO<sub>2</sub> during ascent at 1000 feet per minute*

RABBIT	GROUND LEVEL, O <sub>2</sub>	FINAL O <sub>2</sub>	GROUND LEVEL, CO <sub>2</sub>	FINAL CO <sub>2</sub>	TOLERANCE
27—Control.....	16.9	2.3	26.7	5.5	35,000
26—Control.....	16.7	1.8	36.5	5.0	40,000
13—Control.....	15.5	1.6	37.8	6.2	38,500
14—Control.....	15.0	2.5	32.6	4.3	38,000
46—Control.....	12.9	2.3	38.7	5.8	37,000
47—Control.....	17.1	1.1	41.4	17.8	42,000
Average.....	15.7	1.9	35.6	7.4	38,400
4—Sulfanilamide.....	17.4	2.2	29.1	11.6	42,000
5—Sulfanilamide.....	13.7	0.9	32.2	17.8	43,000
21—Sulfanilamide.....	15.6	1.1	34.7	11.2	45,500
1C-9—Sulfanilamide.....	17.4	1.0	32.3	16.2	41,000
534A—Sulfanilamide.....	15.6	3.3	27.8	17.8	31,000
970A—Sulfanilamide.....	17.8	1.6	21.3	7.5	40,000
C40—Sulfanilamide.....	18.2	2.2	35.7	21.8	36,000
1C-22—Sulfanilamide.....	16.8	0.9	29.6	8.4	45,000
1C-20—Sulfanilamide.....	18.2	1.4	29.0	10.8	46,500
Average.....	16.7	1.6	30.2	13.7	41,100

were still high (averaging 12.8 vol. per cent). Such results suggest strongly an important influence of moderate carbon dioxide retention on the altitude attained.

*The levels of oxygen and carbon dioxide in the venous blood of rabbits during decompression* were only examined on a few occasions. In one rabbit both arterial and venous samples were taken. The arterio-venous differences for oxygen and carbon dioxide at ground level were 8.8 and 4.7 volumes per cent. At 41,000 ft. the arterio-venous oxygen difference had fallen to 1.5 volumes per cent, whereas that for carbon dioxide remained unchanged.

*The levels of oxygen and carbon dioxide in the arterial blood during decompression and their relation to alveolar air* were investigated in dogs. These animals were

examined at ground level, then exposed to a simulated altitude and then both sets of observations were repeated following a large intravenous dose of sulfanilamide. If the control arterial blood carbon dioxide tensions did not coincide closely with those found in alveolar air, the whole experiment was discarded. In those experiments where the results seemed entirely acceptable, no differences were found between these tensions at any altitude, either before or after sulfanilamide. The results of two experiments are summarized in table 2.

TABLE 2  
*Arterial blood-alveolar air CO<sub>2</sub> tension relationships*

	TIME	SAMPLE	PRESSURE*	PLASMA pH	PLASMA CO <sub>2</sub>	PLASMA CO <sub>2</sub> TENSION	ALVEOLAR CO <sub>2</sub> TENSION
D-2							
			<i>mm. Hg</i>		<i>vols. per cent</i>	<i>mm. Hg</i>	<i>mm. Hg</i>
Before sulfanilamide	1128	I	751 (0)	7.35	46.4	37.5	36.0
	1201	II	281 (25,000)	7.56	34.5	16.9	17.1
	117	III	195 (33,000)	7.65	27.1	10.7	10.6
After sulfanilamide— blood level 20 mgm. %	259	IV	751 (0)	7.52	33.0	17.6	15.5
	321	V	281 (25,000)	7.68	28.5	10.5	9.5
	340	VI	195 (33,000)	7.69	28.4	10.4	9.3
	412	VII	751 (0)	7.53	27.8	14.5	14.6
	432	VIII	224 (30,000)	7.66	19.3	7.5	5.6
D-13							
Before sulfanilamide	1112	I	759 (0)	7.41	37.2	25.3	24.4
	1130	II	345 (20,000)	7.46	30.7	18.4	17.2
	1144	III	225 (30,000)	7.47	21.2	12.5	10.4
After sulfanilamide— blood level 25 mgm. %	118	IV	759 (0)	7.36	32.0	24.2	25.4
	140	V	349 (20,000)	7.46	28.6	17.4	14.3
	151	VI	225 (30,000)	7.58	15.0	7.5	7.9
	201	VII	225 (30,000)	7.42	14.8	9.6	8.4
	319	VIII	759 (0)	7.47	25.7	15.3	16.8

\* Numbers in parentheses indicate altitude in feet.

*The possible relationship of methemoglobinemia to altitude tolerance* when repeated doses of sulfanilamide had been employed received some investigation. Wendel (5) states that sulfanilamide does not produce methemoglobin in rabbits or dogs as it does in man (6). Consequently, methemoglobinemia was induced by intravenous injection of sodium nitrite. A number of experiments were made on rabbits in which sodium nitrite injections had reduced the oxygen capacity by 5 to 9 volumes per cent. A reduction of the altitude tolerance by some 3,000 ft. was found, if the standard rate of ascent of 1,000 ft. per minute was employed. This apparently slight effect became more evident if exposures to low oxygen

tensions of longer duration were used. Thus four rabbits received 60 mgm. of sodium nitrite per kilo, were taken to 25,000 ft. and were kept at this simulated altitude for 25 minutes. All four animals died, though such an experience has not harmed a single one of many normal rabbits.

Two dogs were used for additional tests. Repeated tests separated by at least two days were employed. The absolute limits of their altitude tolerances, during an ascent at 1,000 ft. per minute, were respectively 38,000 and 46,000 ft. Two hours after a dose of sodium nitrite of 30 mgm. per kilo had been given intravenously, the concentrations of methemoglobin in their bloods (determined by the loss in oxygen capacity) were equivalent to 13 and 8 volumes of oxygen per 100 ml. of blood. Their altitude tolerances were then 35,000 and 39,000 ft. During these latter tests signs of impairment of function, such as weakness of the limbs, loss of response to bell-ringing, and profound unconsciousness developed somewhat earlier than during the control tests. Even more marked was an earlier development of respiratory responses to anoxia. When methylene blue was injected intravenously (0.2 ml. of a 1 per cent solution was given per kilo of body weight) two hours after such a dose of sodium nitrite, the concentrations of methemoglobin were only equivalent to a loss of 1 and 3 volumes of oxygen per 100 ml. The altitude tolerances were not lowered; they were 42,000 and 45,000 ft.

In all these tests no evidence was found of any permanent damage. The dogs all recovered their normal posture and previous appearance within 5 to 15 minutes.

Methylene blue injections had been also employed after dosage with sodium nitrite in five rabbits. It appeared ineffective, but the later experiments in dogs suggest that the dosage used was probably inadequate.

**DISCUSSION.** The results obtained may be briefly summarized to facilitate discussion. Sulfanilamide given to rabbits in heavy doses increases the resistance of rabbits to low oxygen tensions. If they be exposed to a simulated altitude at a rate of decompression equivalent to an ascent at 1,000 ft. per minute, the altitude they can tolerate is raised about 3,000 ft. This increased tolerance is associated with, and probably dependent on a relative retention of carbon dioxide in the blood. Though there is a fall in the content of carbon dioxide in the blood, it is not nearly as great as that observed in control animals. The question arises whether this retention of carbon dioxide is dependent on the known depression of carbonic anhydrase by the drug.

The effect of the drug, when given under the conditions of these experiments, in raising the altitude tolerance is so definite and consistent that it is clearly established. This is true even though the average limits of tolerance attained by the sulfanilamide-treated animals are by no means outside the limits of individual variation in rabbits. This is in agreement with observations on monkeys made by Fulton (7).

During the altitude tests many of the rabbits showed a head retraction which lasted a few minutes only and was usually seen at about 18,000 to 25,000 ft.

At 18,000 ft. there was also some loss in temperature control which allowed a sudden rise in the skin temperature of the ear (8). During the rapid descent, after the occurrence of gasping or other changes in respiration had caused the ascent to be reversed, a generalized involuntary muscular tremor might develop. This usually persisted for several minutes after ground pressure had been restored and frequently extended into gross convulsive movements. Sometimes the tremors commenced during the last few thousand feet of the ascent. For some unknown reason the tremors and convulsions appeared to be more frequent in animals treated with sulfanilamide.

In rabbits on exposure to decompression the changes in the gaseous content of the blood were often not simple. There was frequently a slight increase in the oxygen content at 20,000 ft., as compared with that found at a slightly lower altitude. This probably depended on the decreased tension of  $\text{CO}_2$  in the lungs and blood. Just before the limit of tolerance was reached the respiration might be depressed and, in consequence, the content of  $\text{CO}_2$  in the blood might rise above its lowest value. In spite of these minor complexities considerable differences were obvious when treated animals were compared with controls. Rabbits dosed with sulfanilamide showed, at ground levels, lowered concentrations of  $\text{CO}_2$  in the blood, a change presumably due to hyperventilation. Such hyperventilation has been observed clinically in man (9). During the ascent the blood content of  $\text{CO}_2$  fell much less in rabbits heavily dosed with sulfanilamide than it did in control animals. When this dosage was lighter the initial lowering of the  $\text{CO}_2$  content of the blood was not seen, but the animals still withstood very high altitudes and also exhibited a fairly high retention of blood  $\text{CO}_2$ .

In the experiments on dogs the carbon dioxide tensions calculable from blood examination were compared with those found in samples of alveolar air. In the more successful experiments no discrepancies were found either before or after sulfanilamide. The control observations preceded those in the presence of the drug. There appeared to be a progressive fall in the content of  $\text{CO}_2$  in the blood during the experiment, and no relative retention of  $\text{CO}_2$  at high altitudes after sulfanilamide could be demonstrated. In such experiments, however, it is difficult to be certain that the blood samples taken are truly representative of blood in equilibrium with the alveolar air drawn, and to prevent the effects of progressive changes in the animal with anesthetic and experimentation.

The question of whether depression of carbonic anhydrase is the cause of the increased altitude tolerance is hard to answer. It could explain the effects observed in the rabbits, since it would allow an abnormal retention of  $\text{CO}_2$  in the blood without any equivalent increase in the alveolar  $\text{CO}_2$  concentration. The increased  $\text{CO}_2$  within the blood would render it more acid and facilitate the unloading of oxygen from the blood. If such an increase in the  $\text{CO}_2$  content of blood was associated with an equivalent increase in  $\text{CO}_2$  in the alveolar air, this  $\text{CO}_2$  would displace sadly needed oxygen (since the nitrogen concentration can only undergo minor alterations) and the uptake of oxygen by the blood would be



impeded. Any incapacity of the blood to liberate  $\text{CO}_2$  from carbonic acid would prevent this rise of alveolar  $\text{CO}_2$ , so that the beneficial effects of  $\text{CO}_2$  in the blood could be obtained, without the deleterious effect of raised  $\text{CO}_2$  in the lungs. For this reason the observations of Binet, Strumza and Voghel (10) that the apnea which terminates acute anoxia occurs just as rapidly with a high blood  $\text{CO}_2$  as with profound acapnia, need not be considered as in direct conflict with the data here reported. The conditions of their experiments were different. Even in the absence of sulfanilamide and its effects moderate retention of  $\text{CO}_2$  may be beneficial in reactions to anoxia according to the observations of Gellhorn (11).

The fact that the  $\text{CO}_2$  tensions calculable from blood examination in dogs are found to agree with those of alveolar air, even after sulfanilamide, gives no evidence that carbonic anhydrase activity is affected, but it does not exclude this explanation. As has been pointed out by Roughton (12), the drug would only slow the chemical processes dependent on this enzyme and not completely inhibit them. Consequently the blood might progressively alter between withdrawal and examination. In the body the pH may have been lower and the carbonic acid concentration in the blood higher than would appear to have been the case as judged by later examination.

One line of evidence would appear to be against this attractive hypothesis. Sulfapyridine is said not to inhibit carbonic anhydrase (13). Eight of the rabbits which had been previously given small doses of sulfanilamide were also tested after a large dose (600 mgm. per kilo) of sulfapyridine. The average altitude reached was the same as that reached after the small therapeutic doses of sulfanilamide and 3,000 ft. higher than in controls.

The experiments so far discussed deal entirely with the acute effects of large or small doses of sulfanilamide on animals. Chronic dosage might produce quite different effects, but since the drug is said not to induce methemoglobinemia in either rabbits or dogs, intravenous injections of sodium nitrite were employed to produce these effects. There was no doubt that the altitude tolerances were lowered, though the animals were able to endure quite high "altitudes." This would appear to be due to the relative insensitiveness of the central nervous systems of these lower species to oxygen lack, as compared with man.

Methemoglobinemia is not presented as a reason why flying personnel should be grounded during sulfanilamide therapy. Sufficiently disabling nervous symptoms have been described by other authors and there is no evidence that these nervous symptoms are in any way related to methemoglobinemia. The rationale of the grounding of air personnel, who are under therapy with sulfanilamide, is not questioned, and it is unlikely to be related to the phenomena that have been described in this paper. On the other hand, conditions may be quite different if air ambulances are employed. The crew might be able to fly with ease at altitudes quite hazardous to their invalid passengers. This difference might be grossly exaggerated if these passengers had received sulfanilamide and had any degree of methemoglobinemia. It would seem advisable, therefore, to give methylene blue to sick or wounded men, who have been so treated, as recommended by Wendel (5), before they are transported by air.

## SUMMARY

1. Sulfanilamide in moderate to heavy doses improves the altitude tolerance of rabbits by some 3,000 feet.

2. This improvement in altitude tolerance is associated with a retention of  $\text{CO}_2$  in the blood, as compared with the untreated animal. The contents average about 33 per cent higher.

3. No significant differences in  $\text{CO}_2$  tension of arterial blood and alveolar air were found at altitude after sulfanilamide administration, so that the cause of the above phenomena is yet unascertained.

4. Methemoglobinemia causes some reduction in altitude tolerance, but tolerance is restored to normal by methylene blue, which hastens the reversion of methemoglobin to hemoglobin.

The writer wishes to thank Dr. A. C. Burton for kind assistance given throughout this investigation, to Miss J. Lang, who made for him some of the examinations of the blood gases, and Miss M. Wheatley, who made the determinations of the concentrations of sulfanilamide in the blood.

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# THE MEASUREMENT OF EDEMA IN THE HEART-LUNG PREPARATION<sup>1</sup>

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The quantitative measurement of edema production in organs such as the heart and lungs is made difficult by the fact that in most experimental situations the weight of the organ cannot be measured before and after periods of changed conditions. When the heart-lung preparation is studied over some hours it is almost invariably found that edema of both heart and lungs occurs (1). It is important to be able to measure the amount of such edema for many reasons, but particularly in order to study changes in water and electrolyte changes in the heart muscle itself.

Several methods suggest themselves as possible means of estimating the edema of organs. *a.* If the organ weight-body weight ratio in the normal animal is known, and varies within sufficiently narrow limits, comparison of observed with predicted organ weight will provide a measure of the increase due to the experimental conditions. If it can be assumed that edema formation is the only process leading to significant weight changes, those changes are obviously a measure of edema production. *b.* If it be assumed that the cells of an organ are and remain chloride-free or nearly so, a calculation by the usual methods, of the extracellular water in excess of the normal, provides a measure of edema (extracellular). *c.* The actual weight changes in an isolated organ, over a period of perfusion, can be measured and the progress of edema formation thus determined. Such a measure involves the assumption that the weight change represents majorly an increase in intra- and extracellular water.

The assumptions underlying these three methods are not inherently improbable, and the measurements themselves can be carried out with considerable accuracy. Another method, *d*, has frequently been used in the past. It consists in a determination of tissue water content. It depends on the assumption that the solids in edema fluid are either negligible or low and constant. It is known that the protein content of edema fluid is variable. Moreover measurement of total solids in tissues is subject to relatively more error, in proportion to expected changes, than are measurements of chloride or total weight. This is true partly because the volatile organic matter which is lost depends upon the time of, and the precise control of the temperature during drying. Altogether it might be predicted, and it is found to be true, that water content is not a reliable measure of edema.

<sup>1</sup> An excerpt from a thesis submitted to the graduate faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1940.

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**METHODS.** Hearts and lungs from apparently healthy mongrel dogs of both sexes were used in these studies. The animals were weighed with an accuracy of 0.1 kgm. or less. Control values for ventricular weight, lung weight and chemical analyses were obtained on 67 "normal" animals sacrificed by rapid bleeding from the carotid under ether anesthesia.

The development of edema was studied in 85 hearts and lungs after various periods following preparation of the Starling heart-lung system. The preparation was removed from the chest and immersed in a constant temperature bath as described by Peters and Visscher (2). At termination of the period of observation the atria were separated from the ventricles by cutting along the atrio-ventricular groove. Blood was removed by blotting with dry filter paper and the fresh ventricular weight determined by means of a torsion balance. The fresh lung weights were similarly determined after trimming off the visible bronchi flush with the lung parenchyma.

The tissue analyses were carried out on duplicate samples of ventricular muscle. Visible fat and connective tissue were trimmed off, and the whole ventricles run twice through a meat chopper, after which suitable samples were taken for analysis. Tissue and blood serum chloride analyses were carried out by method of Van Slyke and Sendroy (3). The water content of blood and tissue was determined by weight differences produced by drying for 48 hours in an oven kept at from 95° to 105°C.

**RESULTS.** The data from all observations on the ventricular weight plotted against body weight are shown in figure 1. The values for lung weight are plotted in figure 2. In each figure the solid points show data for normals and the open circles for heart-lung preparation organs. It is apparent that there is considerable overlapping of values in the case of ventricular weight but that there is virtually complete absence of overlap in the values for lung weight at a given body weight in the two groups. It should be pointed out that the absence of values for organ weights from heart-lung preparations at the higher body weights is due to the fact that no animals above 20 kgm. weight are used for heart-lung preparations because the apparatus employed will not accommodate such large hearts.

The average and extremes of ventricular weight/body weight and lung weight/body weight ratios of normal dogs of various weight ranges and of all the heart-lung preparations are given in table 1. The standard deviation from the mean ratios calculated from the entire normal series was  $\pm 0.121$  per cent of the body weight for the ventricular weight/body weight ratio and  $\pm 0.080$  per cent of the body weight for the lung weight/body weight ratio. The average normal ratios are in reasonable agreement with similar values reported by Stewart (4) and Herrmann (5). A significant variation of these ratios with the body weight is not evident in this series.

Both the average ventricular weight/body weight and lung weight/body weight ratios from the heart-lung preparations are significantly greater than the normal average ratios. Comparison of the average heart-lung and normal ratios indicates that the average increase in weight (edema) of the heart-lung

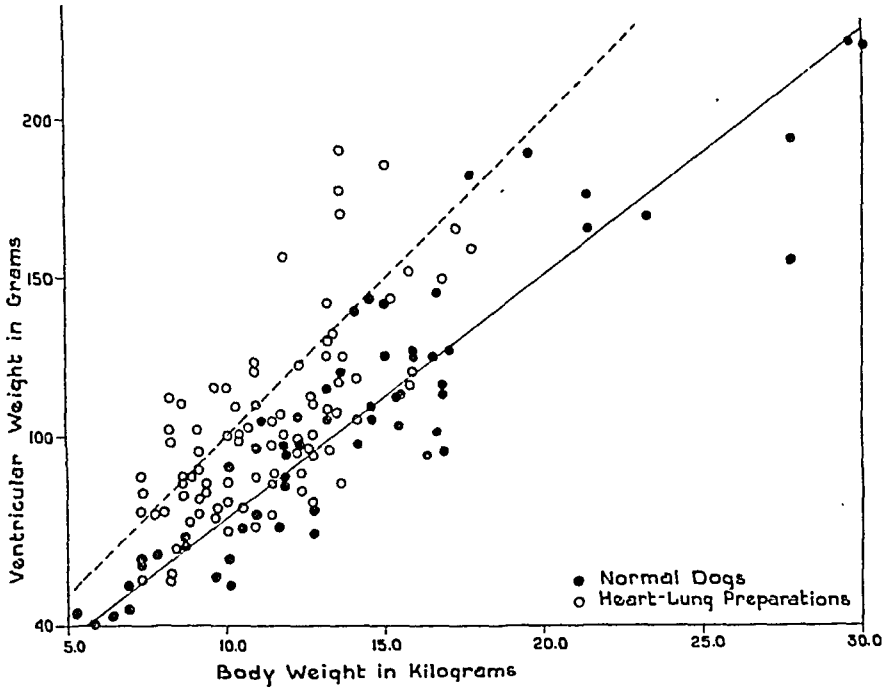


Fig. 1. The relationship of ventricular weight to body weight in normal dogs and in heart-lung preparations. The solid line represents the average ventricular weight/body weight ratio of normal dogs ( $0.758 \pm 0.121$ ). The broken line represents the average normal ratio ( $0.758$ ) plus twice the standard deviation ( $\pm 0.121$ ).

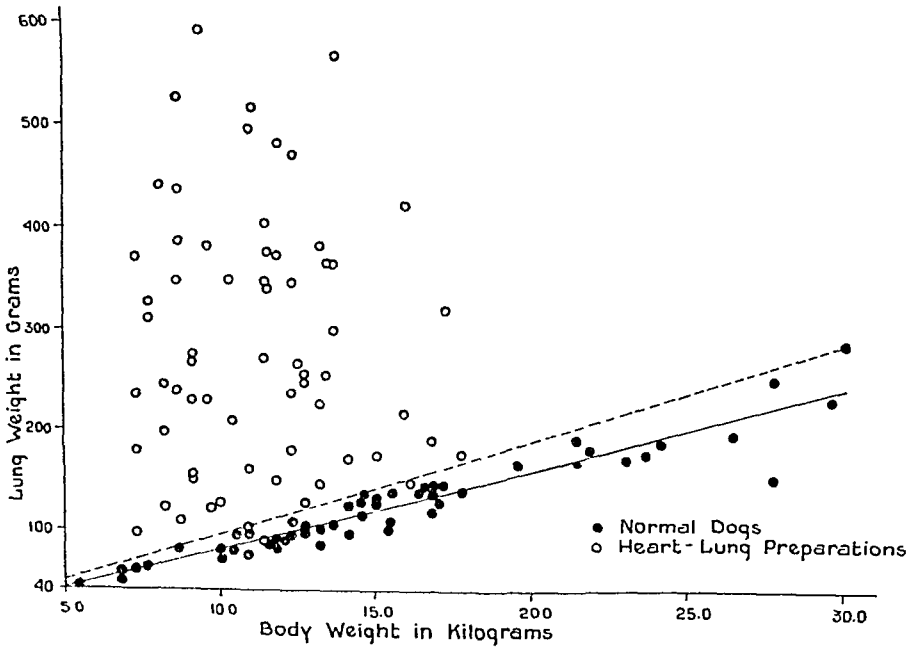


Fig. 2. The relationship of lung weight to body weight in normal dogs and in heart-lung preparations. The solid line represents the average lung weight/body weight ratio of normal dogs ( $0.796 \pm 0.080$ ). The broken line represents the average normal ratio ( $0.796$ ) plus twice the standard deviation ( $\pm 0.080$ ).

ventricles at the end of the preparation was 18 per cent and of the lungs 60 per cent of the final organ weight.

The increase in weight (edema accumulation) of 4 blood-perfused Langendorff dog heart preparations (6) has been recorded continuously for the duration of the perfusion period. An attempt to minimize the variations in the weight of the heart due to variations in the volume of the intravascular perfusion fluid has

TABLE 1  
*Ventricular weight-body weight and lung weight-body weight ratios*

DOG WEIGHT RANGE, KGM.	(NO.) DOG WEIGHT KGM. (EXTREMES)	(NO.) VENTRICULAR WEIGHT GM. (EXTREMES)	(NO.) LUNG WEIGHT GM. (EXTREMES)	PER CENT OF BODY WEIGHT	
				(No.) Ventricles (Extremes)	(No.) Lungs (Extremes)
Normal dogs					
5-10	(17)	(17)	(11)	(17)	(11)
	8.38	61.8	69.6	0.743	0.806
	(5.46-10.90)	(40-96)	(44-93)	(0.530-0.953)	(0.720-0.936)
11-15	(25)	(25)	(21)	(25)	(21)
	13.50	106.6	107.3	0.783	0.798
	(11.10-15.90)	(69.0-142.5)	(82.0-138.0)	(0.543-0.987)	(0.652-0.938)
16-20	(12)	(10)	(11)	(10)	(11)
	17.69	128.7	145.5	0.749	0.819
	(16.35-20.90)	(94.0-189.0)	(119.0-175.0)	(0.575-1.030)	(0.707-0.878)
21-30	(12)	(7)	(11)	(7)	(11)
	25.00	185.0	197.0	0.720	0.785
	(21.36-30.00)	(145.0-223.0)	(150.0-282.0)	(0.523-0.823)	(0.545-0.970)
Complete series averages:	(67)	(59)	(54)	(59)	(54)
	14.92	106.5	125.7	0.758	0.796
	(5.46-30.00)	(40.00-223.0)	(44.0-282.0)	(0.523-1.030)	(0.545-0.970)
Heart lung preparations					
7-18	(85)	(85)	(65)	(85)	(65)
	10.3	105	213	0.920	2.068
	(7.3-17.7)	(54-190)	(91-590)	(0.607-1.390)	(0.780-6.125)

been made by measuring the weight of the preparation at zero perfusion pressures at the beginning and the end of the experiment. During the experiment the perfusion pressure was maintained at constant level. Weight changes due to variations in the volume of the vascular bed are undoubtedly small in comparison to the total changes in weight which were found (6).

Plotting the increase in weight of the heart against the duration of the preparation shows that over 90 per cent of the weight gain occurs during the first 30 minutes. After this initial rapid weight gain the weight of the heart increases

only very slowly for the duration of the preparation. Somewhat similar weight gain curves have been obtained by Ort and Markowitz (14) using the Langendorff rabbit heart preparation.

The heart and blood serum of the perfusion fluid were analyzed for chloride. The edema content of these hearts has been calculated<sup>2</sup> from their chloride content, on the assumptions that the muscle cells retain their "normal impermeability" to chloride and that the edema is extracellular. The average normal ventricular and serum chloride values which were used in the calculations were obtained from a series of normal dogs dispatched by hemorrhage while under ether anesthesia. These values are reported elsewhere (17).

Table 2 shows a comparison of the calculated edema values with the values determined by direct weight measurement. The agreement that was obtained is satisfactory and in all probability within the limits of error of this type of experiment.

TABLE 2  
*Edema formation in blood-perfused Langendorff dog heart preparations*

HEART WEIGHT BY TORSION BALANCE		CHLORIDE CONTENT		FINAL EDEMA CONTENT	
Initial	Final	Heart	Serum	Weight method	Chloride method
grams	grams	mgm./100 grams	mgm./100 grams	grams/100 grams of final heart weight	grams/100 grams of final heart weight
231	374	277	506	39.3	39.1
106	170	287	506	38.7	41.7
134	204	312	550	34.3	41.6
121	157	245	575	22.9	22.7
Average 148	226			33.8	36.3

The edema content of 23 heart-lung ventricles has been calculated from ventricular chloride analyses, and of 33 ventricles from ventricular water contents. A check on the adequacy of these two methods can be gained by comparing the ventricular weight-body weight ratios of normal dogs with the ratios obtained in the heart-lung ventricles with and without correction for edema (table 3). The average ratio of the heart-lung ventricles after correction for edema by the

<sup>2</sup> The derived equation for the calculation of edema by the "chloride space method":

$$E = 100 [Cl_r - Cl_n(Cl_s/Cl_{ns})] \div [Cl_s - Cl_n(Cl_s/Cl_{ns})]$$

$E$  = Edema content in grams per 100 grams of final ventricular weight

$Cl_r$  = Final ventricular chloride in milligrams per 100 grams of fresh tissue

$Cl_n$  = Average normal ventricular chloride in milligrams per 100 grams of fresh tissue

$Cl_s$  = Final serum chloride in milligrams per 100 grams of serum

$Cl_{ns}$  = Average normal serum chloride in milligrams per 100 grams of serum.

A similar relation can be used to calculate edema from tissue water analyses:

$$E = 100 (H_2O_r - H_2O_n) \div (H_2O_E - H_2O_n)$$

$E$  = Edema content in grams per 100 grams of final ventricular weight

$H_2O_r$  =  $H_2O$  content of ventricles in grams per gram of fresh tissue

$H_2O_n$  =  $H_2O$  content of normal ventricles in grams per gram of fresh tissue

$H_2O_E$  =  $H_2O$  content of the edema fluid in grams per gram of edema fluid.

chloride method is approximately normal. The average ratio is still considerably above normal after correction for edema by the water content method, indicating that this method does not adequately correct for edema.

Reference to figure 2 shows that a reasonably accurate value for the amount of lung edema occurring in a preparation can be determined by comparing the

TABLE 3  
*Average ventricular weight-body weight ratios*

	NORMAL DOG VENTRICLES	HEART-LUNG VENTRICLES	HEART-LUNG VENTRICLES AFTER CORRECTION FOR EDEMA BY		
			H <sub>2</sub> O method	Chloride method	Lung edema method
Number.....	(59)	(85)	(33)	(23)	(63)
Average.....	0.758	0.920	0.852	0.732	0.715
Extremes.....	(0.530-0.955)	(0.607-1.590)	(0.599-1.292)	(0.585-0.908)	(0.485-1.000)

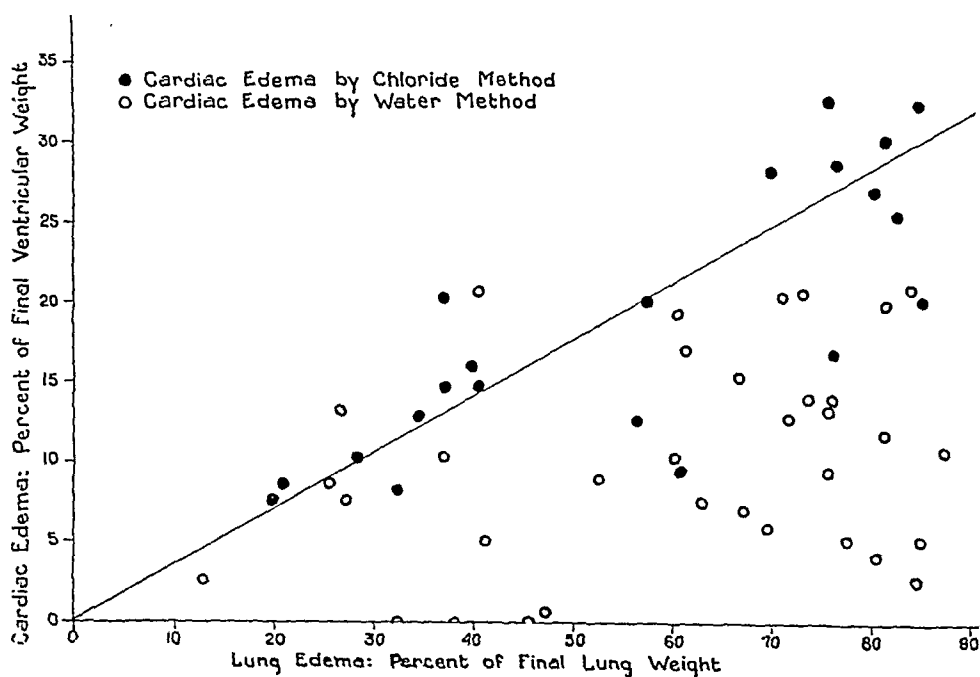


Fig. 3. The relationship between lung and cardiac edema in the heart-lung preparation. The solid line is an arbitrary correlation line which was used to approximate the cardiac edema of heart-lung ventricles of unknown chloride content.

final lung weight with the initial lung weight predicted from the average normal lung weight/body weight ratio.

In heart-lung preparations, edema of both organs presumably results from similar causes, such as toxic substances in the blood and increased venous pressure. A correlation was therefore sought between cardiac edema (chloride method) and lung edema (weight measurement). The results are shown in figure 3. A positive correlation is evident between the relative amounts of



lung edema and of cardiac edema as calculated by the chloride method. A correlation between lung edema and the cardiac edema as calculated from water analyses is not evident; an additional indication that the magnitude of edema formation can not be measured adequately by means of water analyses.

Using the correlation found between lung edema and "cardiac chloride edema," the cardiac edema of 65 heart-lung ventricles has been estimated from the lung edema which occurred in these preparations. The adequacy of this method for the estimation of cardiac edema is also indicated in table 3. The average heart weight to body weight ratio obtained after edema correction by this method is somewhat below the normal ratio, indicating an overcorrection for the edema. This overcorrection was expected since several experiments, in which the cardiac-lung edema correlation was far removed from the other experiments, were omitted when the arbitrary correlation line was drawn through the experimental points. In these experiments which have been omitted it has been observed that the lung edema occurred early in the preparation and was of a very rapidly developing massive type which caused an early death of the preparation. This type of lung edema is associated with relatively very small amounts of simultaneously occurring cardiac edema and perhaps the underlying mechanism is of a different type than that which causes the pulmonary edema usually seen in this preparation.

**DISCUSSION.** Obvious lung edema has been known to occur in most heart-lung preparations but quantitative studies of this phenomenon have previously been lacking. The cause of this lung edema is not clear. The earlier investigators (7, 8, 9, 10) included such physical factors as high pulmonary arterial and venous pressures, high rates of blood flow, and decreased osmotic pressure, cell volume, and viscosity of the blood as determining causes of pulmonary edema of this type. More recently Lambert and Gremels (11) and Newton (12) have shown that some toxic factor in shed blood which appears and increases as the blood stands is the chief causative factor of the pulmonary edema. Code, Evans and Gregory (13) demonstrated that this toxic factor is in all likelihood not histamine.

The present study shows that cardiac edema also occurs to a greater or less degree in all heart-lung preparations, presumably due to the action of the same factors which result in the accumulation of edema fluid in the lungs. Ort and Markowitz (14, 15) have shown that hearts perfused through the coronary arteries gain from 20 to 150 per cent of their original weight during the first hour of perfusion depending upon whether heparinized blood or Locke's fluid was used as the perfusing fluid. Peters and Visscher (2) have reported increases of 20 per cent in the volume of ventricular musculature of the heart-lung preparation during a period of 25 minutes under special conditions.

The average amount of cardiac edema which has been found to occur in the heart-lung preparation is of such magnitude as seriously to compromise any experimental results obtained from this preparation which are expressed in terms of the final fresh or dry heart weight. This statement probably applies to most isolated perfused preparations.

The inadequacy of the correction for edema on the basis of tissue water determinations arises from the fact that the edema fluid in this type of preparation has a relatively high dry weight content which cannot be readily determined. Water content determinations on the fluid which exudes from obviously edematous heart-lung hearts upon standing give average values of 90 per cent as compared to the average plasma value of 93 per cent. Such hearts not uncommonly have a normal water content although chloride analyses indicate that over 20 per cent of their weight is edema fluid.

The results indicate that the cardiac edema which occurs in the heart-lung preparation can be estimated with reasonable accuracy as an increase in the "extracellular water"<sup>3</sup> content. Therefore this edema appears to be for the most part extracellular. Permeability of dead or injured muscle cells to chloride should cause a positive error in the extracellular water calculation, but intracellular edema, which would not be included in edema calculated from chloride analyses, would tend to cancel this error.

Evidence from potassium, sodium, chloride and water analyses of heart-lung ventricles (18) indicates that their intracellular electrolyte and water pattern is essentially normal. The "extracellular water" content of heart-lung ventricles is, however, much larger than normal, indicating that the site of edema formation is extracellular. The factor or factors which cause the marked alteration in capillary permeability resulting in edema formation in the heart-lung preparation evidently do not affect the existence of the electrolyte concentration gradients which are normally present across the cardiac muscle cell membrane.

The external ventricular volume of the majority of the heart-lung preparation hearts was recorded throughout the experiment by means of a Henderson-type cardiometer. It is the opinion of the authors that this procedure considerably increases both cardiac and lung edema formation in this preparation.

#### SUMMARY

1. The average ventricular weight/body weight, and lung weight/body weight ratios from eighty-five heart-lung preparations have been compared with similar ratios from normal dogs. The differences found in the average ratios indicate that on the average 18 per cent and 60 per cent of the final heart-lung ventricular and lung weights respectively are edema fluid.

2. Satisfactory agreement was found between the edema calculated from cardiac chloride analyses and the edema determined by the weight increase of four blood-perfused Langendorff dog hearts.

3. The average ventricle weight/body weight ratios of 23 heart-lung hearts corrected for edema on the basis of chloride analyses was not significantly different from normal.

4. The edema content of heart-lung hearts cannot be adequately corrected for edema on the basis of tissue water content. Concentrations of tissue con-

<sup>3</sup> "Extracellular water" has been used (16) as a non-committal term for the calculated chloride space, since this calculated value is actually neither the "chloride space" or "extracellular space" but only a good approximation of these two phases of tissue.

stituents of this or similar preparations are of little significance if expressed on a dry or a wet weight basis.

5. In the majority of heart-lung preparations a positive correlation exists between the relative magnitudes of cardiac and lung edema which occurs. This constitutes an indication that similar factors are responsible for the edema formation in the two organs.

6. The major part of the edema fluid which accumulates in heart-lung hearts appears to be extracellular in position.

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# ELECTROLYTE AND WATER CONTENT OF THE VENTRICULAR MUSCULATURE OF THE HEART-LUNG PREPARATION WITH SPECIAL REFERENCE TO THE EFFECTS OF CARDIAC GLYCOSIDES<sup>1</sup>

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The studies of Harrison and his co-workers (1, 2) have demonstrated that heart failure in humans is associated with changes in the electrolyte composition of the cardiac musculature. The potassium content of hearts from patients dying of heart failure was found to be decreased. A similar change was found in the hearts of dogs receiving toxic doses of digitalis (3). More recent studies have demonstrated that toxic doses of digitalis cause a decrease in the potassium content of striated muscle (4), the ventricular musculature of the heart-lung preparation (5), the Langendorff perfused rabbit heart (6), and isolated strips of the ventricular musculature of the turtle (7). The reported effects of "therapeutic doses" of digitalis bodies on the potassium content of heart muscle are controversial. Calhoun and Harrison (3) reported a slight decrease of questionable significance in the cardiac potassium content of the intact dog, the blood potassium findings of Wood and Moe on the heart-lung preparation (5, 8) indicated a loss of potassium from the heart and/or the lungs, Wedd (7) could demonstrate no change in potassium content of the heart of the intact cat or in isolated strips of the turtle heart, Hagen (6) found the potassium content of Langendorff perfused rabbit hearts was increased after "therapeutic doses" of Lanatoside C, and Boyer and Poindexter (9) found that therapeutic digitalis dosage increased the potassium content of the hearts of intact cats.

Results of potassium, sodium, chloride and water analyses of the ventricular musculature of failing heart-lung hearts with and without treatment with cardiac glycosides are reported in this paper.

**METHODS.** The heart-lung preparations were set up as described by Peters and Visscher (10) so that oxygen consumption and external diastolic volume could be recorded continuously, and the measurements necessary for the calculation of the external work and hence the external mechanical efficiency of the preparation could be made at suitable intervals.

The preparation and analysis of the ventricular musculature were carried out as described by Wood and Moe (11) and Wood (12).

**RESULTS.** The ventricles of forty heart-lung preparations receiving none or

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various doses of digitalis glycosides<sup>2</sup> have been analyzed for potassium and water. Some of these hearts have also been analyzed for sodium and chloride.

The average values and the range of variation in the potassium and water analyses are given in table 1. The "digitalis hearts" are subdivided according to whether they received "toxic" or "therapeutic" doses of a digitalis glycoside. In this paper toxic and therapeutic doses are defined in reference to the heart-lung preparation in accordance with Moe and Visscher (13). A toxic dose of a digitalis glycoside is one which produces cardiac arrhythmias in the heart of the heart-lung preparation. A therapeutic dose is one which will increase the external mechanical efficiency of the heart-lung without the production of cardiac arrhythmias within a period of 150 minutes after administration of the drug.

TABLE 1  
*Heart-lung ventricles, average values*

(NO.) HEARTS OF	WATER CON- TENT PER 1000 GRAMS FRESH TISSUE	POTASSIUM CONTENT PER		DURATION OF PREPARATION	EDEMA CONTENT OF VENTRICLES AS CALCULATED BY		K CONTENT PER 1000 GRAMS "EDEMA CORRECT- ED"†, FRESH TISSUE
		1000 grams fresh tissue	100 grams of dry weight		Lung edema method*	H <sub>2</sub> O content method*	
	grams	mM	mM	minutes	grams per cent	grams per cent	mM
(15) Normal dogs	783 (766-799)	81.5 (73.4-87.0)	37.4 (31.6-40.0)				81.5
(12) Control Heart-lungs	799 (784-825)	71.6 (62.1-85.9)	35.6 (32.3-42.3)	183 (104-255)	20.0 (12.5-28.7)	10.6 (0.65-23.8)	88.5
(7) Therapeutic dose Heart-lungs	799 (781-812)	63.1 (49.2-75.0)	31.6 (27.2-33.8)	249 (147-316)	25.0 (15.7-32.2)	10.6 (0.0-18.8)	83.2
(15) Toxic dose Heart-lungs	795 (781-810)	59.2 (44.6-71.9)	28.2 (20.6-33.6)	185 (92-330)	21.5 (7.6-30.2)	7.8 (0.0-17.5)	72.4

\* Wood and Moe (1941).

† Calculated by lung edema method (Wood and Moe, 1941).

Without chloride analyses there is no adequate means of correcting for edema in heart-lung hearts. Table 1 includes the amount of cardiac edema as calculated by the water content and lung edema methods, the only available means of estimating the edema content in this group of hearts since they were not analyzed for chloride (11). The actual cardiac edema probably correlates more closely with the calculated edema based upon the simultaneously occurring lung edema (11). Therefore the ventricular potassium content has been computed on the basis of edema corrected (lung edema method) fresh tissue weight in an effort to gain a better idea of the exchanges occurring in the actual cardiac tissue. The inadequacy of this correction is shown by the fact that these calculations (table 1) show higher potassium concentrations in control and thera-

<sup>2</sup> The pure glycosides of *Digitalis lanata*, Lanatosides A, B, or C, were used in the majority of these experiments.

peutic dose hearts than are obtained with normal hearts. Average edema values as obtained by this method have been shown to overcorrect for edema (11).

In spite of the difficulty of interpretation due to cardiac edema, several definite indications can be drawn from table 1: 1, heart-lung ventricles have an increased water content, presumably due to the accumulation of edema fluid; 2, with the doses used in these experiments the magnitude of this increase is apparently not affected by digitalis glycosides; 3, all heart-lung ventricles have a decreased potassium concentration compared to the normal dog heart when concentrations are expressed on either a fresh tissue or a dry weight basis; 4, hearts receiving toxic doses of digitalis glycosides show a much greater decrease in potassium concentration than the control heart-lung hearts; this decrease is much too large to be explained on the basis of edema content; 5, hearts receiving therapeutic doses of digitalis glycosides show a greater decrease in potassium concentration than do control hearts. However, it must be noted that the average duration of the preparation was about one-third greater in the therapeutic dose digitalis experiments than in either the controls or the toxic dose experiments. This greater time would favor spontaneous potassium loss and edema formation. The correction for edema leaves a small apparent loss of potassium in comparison with control hearts which is slightly less than one-third that found with toxic doses of digitalis glycosides.

More decisive information can be derived from the hearts which were analyzed for sodium and chloride in addition to the potassium and water analyses. The average values and range of variation of these analyses are given in table 2. The wet and dry weight potassium and water concentration changes in this group of hearts are similar to those shown in table 1. The chloride concentration in heart-lung ventricles is increased; the magnitude of this increase is apparently not significantly affected by the doses of digitalis glycosides used in these experiments. The sodium concentration in heart-lung ventricles is likewise increased; the magnitude of this increase is significantly larger in preparations which received a toxic dose of a digitalis glycoside.

The amount of cardiac edema occurring in this group of hearts was calculated from both the sodium and chloride analyses as described by Wood and Moe (11). A knowledge of the edema content of the heart makes possible the calculation of the approximate exchanges which occurred in the actual ventricular musculature. The average results of some of these calculations are given in table 3.

The average edema contents of the control heart-lung ventricles as calculated either from the sodium or chloride concentrations were not significantly different and amounted to 17.5 grams per cent of the final ventricular weight. The potassium content when calculated on the basis of edema corrected fresh tissue was not significantly different from normal, 80.0 mM per kilo as compared to—81.5 mM per kilo, the average normal figure. The gains in chloride and sodium were "equivalent" when calculated on the basis of their concentrations in serum; hence the increase in sodium and chloride content can be explained on the basis of edema formation alone.

The average edema content of the toxic dose heart-lung ventricles was 25.3

grams per cent on the basis of chloride analyses. The potassium content when calculated on an edema corrected fresh tissue basis was significantly reduced, 66.1 mM per kilo as compared to 81.5 mM per kilo, the average normal value.

TABLE 2  
*Heart-lung ventricles, average values*

(NO.) HEARTS OF	WATER CONTENT PER 1000 GRAMS FRESH TISSUE	CONCENTRATION IN mM PER :						MINUTES DURATION OF PREP- ARATION
		Potassium		Sodium		Chloride		
		1000 grams fresh tissue	100 grams of dry weight	1000 grams fresh tissue	100 grams of dry weight	1000 grams fresh tissue	100 grams of dry weight	
(15) Normal dogs	<i>grams</i> 783 (766-799)	81.5 (73.4-87.0)	37.4 (31.6-40.0)	35.3 (32.7-39.1)	16.3 (14.3-18.9)	29.0 (24.8-33.0)	13.4 (11.7-15.6)	
(5) Control Heart-lungs	810 (784-835)	66.7 (60.7-75.1)	35.1 (32.3-37.7)	54.8 (47.5-64.8)	28.8 (23.4-39.3)	46.2 (43.5-51.9)	24.3 (20.1-31.3)	152 (105-182)
(8) Toxic dose Heart-lungs	810 (791-852)	50.8 (44.6-66.0)	25.6 (21.8-30.4)	75.6 (52.6-92.1)	40.3 (25.6-57.0)	52.1 (40.3-65.6)	27.4 (19.7-43.2)	207 (92-330)
Average serum values								
Normal dogs	937	4.69		141		113		
Control Heart-lungs		3.82		144		119		
Toxic dose Heart-lungs		5.72		143		117		

TABLE 3  
*Calculated electrolyte and water exchanges of heart-lung ventricles, average values*

(NO.) HEARTS OF	CALCULATED EDEMA CONTENT FROM:		POTASSIUM CON- TENT ON BASIS OF "EDEMA CORRECTED" FRESH TISSUE	Cl GAIN	Na GAIN	Na "SERUM EQUIVA- LENT" OF Cl GAIN	Na GAIN IN EXCESS OF "SERUM EQUIVALENT" OF Cl GAIN	K GAIN
	Chloride content	Sodium content						
	<i>grams</i>	<i>grams</i>	<i>mM per 1000 grams of fresh tissue</i>				<i>mM</i>	<i>mM</i>
(5) Control Heart-lungs	175	173	80.0	20.9	25.0	25.2	-0.2	-1.5
(8) Toxic dose Heart-lungs	253	370	66.1	29.6	48.7	36.3	12.4	-11.6

The gain in sodium was in excess over the "serum equivalent" of the chloride gain. This excess amounted to 12.4 mM per kilo of fresh tissue, and is presumably due to the entrance of sodium into the muscle cell in exchange for the lost po-

tassium. The loss in potassium was 11.6 mM per kilo of fresh tissue, approximately the chemical equivalent of the sodium gain.

A better concept of the actual cardiac muscle cell exchanges which occurred can be gained by calculating the "extracellular water"<sup>3</sup> content of these heart-lung hearts. This makes possible a calculation of the approximate intracellular space composition as described by Hastings and Eichelberger (14).

In calculating the "extracellular water" of the heart-lung ventricles it was assumed that the extracellular fluid had the same protein content as the serum

TABLE 4

*Comparison of the extracellular and intracellular composition of normal dog ventricles, control heart-lung ventricles, and heart-lung ventricles from preparations which received a toxic dose of a digitalis glycoside*

	GRAMS H <sub>2</sub> O/ KGM. OF FRESH TISSUE	POTASSIUM	SODIUM	CHLORIDE
15 normal ventricles				
		mm. per kgm. of H <sub>2</sub> O		
Whole ventricle.....	783	104	45.2	37.1
Extracellular space: (23.2 grams per cent)....	990	4.74	145	127
Intracellular space: (76.8 grams per cent)....	721	145	3.77	0.0
5 heart-lung ventricles—no drug				
Whole ventricle.....	810	81.4	67.6	57.0
Extracellular space: (38.8 grams per cent)....	937	4.08	154	127
Intracellular space: (61.2 grams per cent)....	729	146		0.0
8 heart-lung ventricles—toxic doses of a digitalis glycoside				
Whole ventricle.....	810	62.7	93.3	64.3
Extracellular space: (44.6 grams per cent)....	937	6.10	153	125
Intracellular space: (55.4 grams per cent)....	710	122	29.9	0.0

(11) and therefore Donnan factor corrections could be neglected. The relation then becomes:

$$\text{Per cent extracellular water gram} = \frac{100 \text{ (mM per kilo ventricular chloride)}}{\text{(mM per kilo serum chloride)}}$$

The average results of these calculations are given in table 4. The "extracellular water" of heart-lung ventricles is significantly increased over normal due to edema formation which is extracellular in position. The differences in the "extracellular water" values for the control and the toxic dose heart-lung ventricles are probably due to the longer duration of the toxic dose preparations (table 2) rather than to an effect of the digitalis glycoside.

<sup>3</sup> "Extracellular water" has been used (15) as a non-committal term for the calculated chloride space, since this calculated value is actually neither the "chloride space" nor "extracellular space" but only a good approximation of these two phases of tissue.



Intracellular water, potassium, sodium and chloride content of control heart-lung ventricles is apparently not significantly different from the normal ventricle. The intracellular composition of the toxic dose ventricles, however, shows definite and significant variations from the controls and normal ventricles. The intracellular potassium content is significantly decreased; 122 mM per kilo of water as compared to 145 mM per kilo of water, the average normal value. The intracellular sodium content is increased. The loss of intracellular potassium is apparently an exchange with an equivalent amount of sodium, since the average increase in intracellular sodium content of 26 mM per kilo of water is approximately chemically equivalent to the corresponding intracellular potassium loss of 23 mM.

**DISCUSSION.** The bearing of these cardiac electrolyte studies on heart failure and digitalis action are of some interest. The uniformity and magnitude of the cardiac edema which was found to occur in these preparations lends some support to Rühl's contention (16) that cardiac edema is at the basis of heart failure in the heart-lung preparation. However, since digitalis glycosides reverse the processes of failure and yet do not decrease edema, it is equally probable that the edema is an independent concomitant of spontaneous failure.

The finding that untreated failing heart-lung hearts lose little if any intracellular potassium does not agree completely with the results obtained in human heart failure (1, 2). This may result from the fact that failure in the heart-lung heart is much more rapid than that usually seen in clinical heart failure. It should be pointed out that chloride analyses were not carried out in the human heart studies so that calculations of the increase in extracellular space could not be made. Most types of clinical heart failure are associated with increases in the amount of connective tissue and of varying degrees of edema in the heart muscle; until these factors are determined results indicating a decrease in cardiac muscle cell potassium should be accepted with reservations. The fact that nearly all cardiac patients are treated with digitalis may also contribute to the decrease in cardiac potassium concentration which has been reported for such patients.

The electrolyte studies on the heart-lung ventricles receiving toxic doses of digitalis glycosides are in essential agreement with previous results obtained with preparations of other types. The sodium and chloride analyses carried out in this study have added additional information not available in previous studies. It is of interest that the ventricular muscle electrolyte exchanges which were found to result from digitalis action are very similar to the exchanges which occur in striated muscle during activity.

Due to cardiac edema the results concerning the effects of therapeutic doses of digitalis on the potassium content of the heart-lung ventricle are not as conclusive as could be desired. It can be definitely concluded, however, that therapeutic doses of digitalis did not cause an increase in the ventricular potassium content of this preparation. Actually the evidence gives some indication that "therapeutic dose hearts" had a decreased potassium content. This inability to confirm the findings of Boyer and Poindexter (9) on the intact cat and the findings of Hagen (6) on the Langendorff perfused rabbit heart may be due to the dif-

ferences in the preparations used. It may be significant that the average normal potassium content for the cat heart as given by Boyer and Poindexter is 17.0 mM per kilo of fresh tissue lower than the values reported from other laboratories (17, 7, 18).

#### SUMMARY

Studies of the electrolyte and water content of the heart-lung ventricle are reported.

The ventricles of the untreated (failing) heart-lung heart have: 1, an increased water content; 2, an increase in the wet and dry weight sodium and chloride concentrations; 3, a decrease in the wet and dry weight potassium concentration. Correction for edema and calculation of the "extracellular water" on the basis of chloride analyses indicate that: 1, the intracellular water and electrolyte content of the untreated heart-lung ventricles is not significantly different from normal ventricles; 2, the average edema content is 17.5 grams per cent of the final ventricular weight and is extracellular in position; 3, the increase of sodium and chloride content of these hearts can be accounted for on the basis of the extracellular edema formation.

The ventricles of heart-lung preparations which received therapeutic doses of a digitalis glycoside have: 1, an increased water content which is comparable to the control heart-lung ventricles; 2, an apparent decrease in potassium content which is larger than that found in the control heart-lung and does not appear to be accountable for on the basis of an increased edema content alone.

The ventricles of heart-lung preparations which received toxic doses of a digitalis glycoside show significant changes in their intracellular electrolyte compositions. The intracellular potassium content is decreased apparently in exchange for sodium since there is approximately a chemical equivalent increase in intracellular sodium content. The doses of cardiac glycosides used in these experiments did not significantly affect the water gain or edema formation which occurs in heart-lung ventricles.

The unphysiological factor or factors which are responsible for cardiac edema formation in the heart-lung preparation must exert their chief effect on the capillary membrane, since the electrolyte concentration gradients across the muscle cell membrane and the intracellular water content of untreated heart-lung hearts are apparently normally maintained.

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## THE NORMAL PNEUMOCARDIOGRAM

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A simple method for obtaining the pneumocardiogram using an ordinary pneumograph was described in a previous paper (Blair and Wedd, 1939). The principal purpose of that study was the calibration of the record in order to determine the excess of arterial blood which left the chest during the early part of systole, not compensated for by venous return. The observations now reported relate the pneumocardiogram, through the electrocardiogram and heart sounds, to venous and arterial pulses and to the mechanical events of the heart cycle.

**EXPERIMENTAL METHODS.** The pneumogram of the chest was obtained by placing the pneumograph around the subject's chest at the level of the 5th or 6th ribs. It was connected by rubber tubing to a Frank segment capsule covered with a rubber membrane. A concave mirror of 1 m. radius of curvature placed on the membrane permitted its movements to be recorded photographically by means of a light beam. The segment capsule was placed at the level of the optical system of the string galvanometer which was used to record the electrocardiogram. A small leak through a capillary tube was left in the pneumograph system so that the light beam would return to the camera when the breath was held. When a more accurate wave form was desired the leak was stopped after the light beam returned to the camera. Simultaneity of pneumocardiogram and electrocardiogram was determined by tapping the subject's arm while it was lying across his chest with both recording instruments attached. In this way it was found that the lag in the pneumograph system was less than 0.01 sec. and no correction for simultaneity was considered necessary. Arterial and venous pulses were recorded with the same apparatus, using a receiving tambour instead of the pneumograph. The abdominal pneumogram was recorded by placing the pneumograph around the abdomen at the level of the umbilicus. Neck and thigh pulses were also obtained by use of the pneumograph. For consistency the records from parts of the body other than the chest have been called pneumograms when they were obtained by means of the pneumograph. With normal subjects, placing of the pneumograph at different levels has been found to change only the magnitude and not the direction of the records. In the case of the chest for example, the movements of a light beam reflected from a mirror on the sternum show volume changes similar to those recorded with the pneumograph. The two sides of the chest, however, do not usually move exactly together. Heart sounds were recorded by means of a crystal microphone, amplifier and oscillograph. Pneumograms may be recorded electrically. An example of this type of record is given in figure 8B.

The natural frequency of the pneumograph used was about 12 cycles per second and that of the Frank capsule 2 or 3 times as great. The natural fre-

quency of the chest or abdomen is probably much lower than that of the pneumograph. It is to be expected, therefore, that the apparatus will record accurately volume changes of the part of the body to which it is applied but the body movements will overshoot the forces impressed by the action of the heart or the circulating blood. Consequently for the most part it will be possible to derive from the records accurate information about only the onset of cardiac and circulatory events and not about their course.

**EXPERIMENTAL RESULTS.** *Heart sounds and chest pneumograms.* In figure 1A is given a record of the heart sounds, the electrocardiogram and the thoracic pneumocardiogram. Small vibrations will be seen in the pneumogram during and following the Q-R-S interval. These are associated with isometric contraction and the beginning of ejection. About 0.14 sec. after the beginning of Q-R-S the pneumogram rises rapidly. This records the rapid collapse, *B*, of the

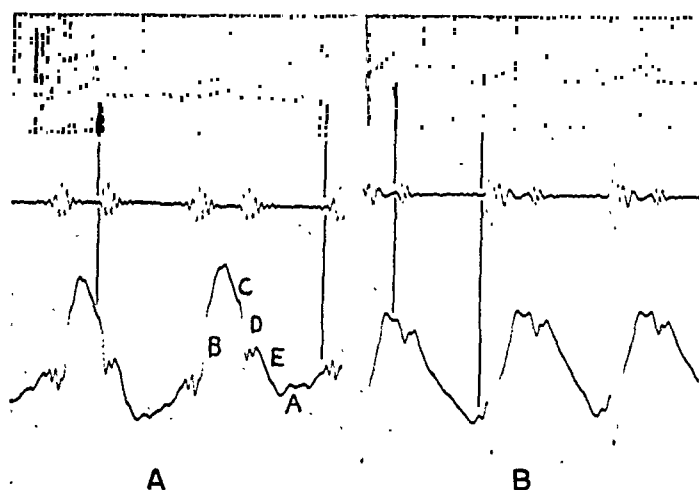


Fig. 1A and 1B. Pneumograms of the chest of two different subjects, with heart sounds and electrocardiograms. An upward movement in the pneumogram indicates a decrease in chest volume. Time marks at 0.04 sec. intervals.

chest due to the outflow of arterial blood. Near the peak of the T wave the collapse reaches its maximum and the chest then expands because the venous return now exceeds the arterial output. The time of the maximum and the form of the wave in this region vary in different subjects and in the same subject in different positions. These points will be discussed later. Corresponding to the beginning of the second sound there is at *C* a small change in slope in the pneumogram which is taken to indicate the closure of the semi-lunar valve. The mechanical unimportance of this event is a tribute to the efficiency of the aortic valve. Following the valve closure is a steep descent of the record, *D*, ending in a plateau with small vibrations. This large movement is considered to be due to relaxation of the ventricles. The reason for ascribing this event to ventricular relaxation is that it just follows the second sound, at which time relaxation is expected. The movement is too late to represent aortic valve closure by a backward movement of the blood in the aorta for that would have to occur before

the second sound and it is too early to be due to ventricular filling. Table 1 shows the relation of the relaxation wave to the beginning of the second sound in a series of subjects. Other reasons for believing this event to be ventricular relaxation will be adduced later. The rapid descent, *E*, following this plateau is due to rapid inflow to the chest of venous blood after the opening of the A-V valves. Following the rapid inflow of venous blood in this particular subject, the thoracic volume reaches its maximum late in diastole and then recedes slightly before auricular contraction occurs at *A*.

It will be seen that the first heart sound begins very soon after isometric contraction begins and that it continues until the large collapse movement of the chest is over. It seems likely, therefore, that in interpreting the low frequency components of the first sound the thoracic wall movements and the factors giving rise to them must be taken into consideration.

The second heart sound lasts through ventricular relaxation and the small vibrations immediately after it. These events probably contribute to the second sound.

TABLE 1

*The intervals in seconds between the peak of the T wave and the second heart sound and the beginning and end of ventricular relaxation in a group of normal subjects*

2ND SOUND	RELAXATION BEGINS	RELAXATION ENDS	2ND SOUND	RELAXATION BEGINS	RELAXATION ENDS
0.05	0.08	0.10	0.13	0.13	0.16
0.02	0.04	0.09	0.08		0.20
0.10	0.14	0.16	0.07	0.09	0.13
0.10	0.12	0.16	0.10	0.13	0.16
0.10	0.13	0.15	0.09	0.09	0.13

Figure 1B is a similar record from another subject. In this instance, collapse of the chest is preceded by a well-marked expansion or apex thrust. The notch just beyond the summit occurs at the time of aortic valve closure and marks this event more distinctly than in the preceding case. Relaxation of the ventricles gives a smaller excursion and venous return during diastole is slower, as shown by the more gradual expansion of the chest back to the baseline. It should be observed that the movements in the pneumogram may be of two kinds. The general trend of the wave at any time is determined by whether or not the thorax is, on the whole, gaining or losing blood. Mechanical events within the thorax such as rotation of the heart and isometric contraction or relaxation may superimpose vibrations without causing net volume change. In normal subjects, for example, ventricular relaxation appears initially to cause rapid expansion of the chest but on the whole during this event the net volume change is probably only that determined by blood movements.

*Venous pulse and chest pneumogram.* Figure 2A shows a venous pulse from the neck with the thoracic pneumocardiogram. The waves of the venous pulse are marked *a*, *c*, and *v*, following the terminology of Mackenzie. It will be seen in the pneumogram that a small expansion of the chest begins with the P wave.

This is probably due to auricular contraction. The chest collapses somewhat at the time the neck swells to give the *a* wave, indicating some venous regurgitation. The *c* wave is not well enough marked in this record to relate it to the pneumogram. It will be seen that the downstroke of the pneumogram attributed to ventricular relaxation is followed by the rapid rise of the *v* wave. It is concluded from this that the rise of the *v* wave is due to an effect of the relaxing ventricles on the large veins and auricles. The rapid expansion of the chest, *E*, due to rapid venous return consequent to the opening of the A-V valves, begins about 0.04 sec. before the downstroke of the *v* wave.

Figure 2B is a venous pulse and a chest pneumogram from another subject. The pneumogram in this case differs in that it has two maxima. It is thought that the expansion of the thorax between these peaks is due to the venous return being larger during this phase of systole than in the subject of figure 2A. The re-

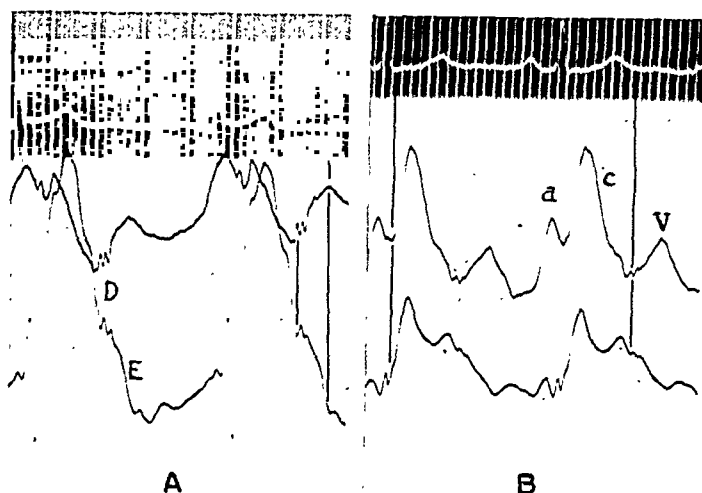


Fig. 2A and 2B. Chest pneumograms from two different subjects—lowest curves in figures, with venous pulses from the neck. An upward movement in the pneumogram indicates collapse of the chest, while an upward movement in the pulse indicates an increase in volume. Time marks at 0.04 sec. intervals. The subject of figure 2A is the same as 1B.

lations of the other events are similar to those in 2A and it can be seen that the *c* wave begins to rise at about the same time the chest begins to collapse.

It will be seen in figure 2A in which the *c* wave is small that the lowest venous pressure occurs about the end of ventricular systole. On the basis of cardiodynamics alone, it would be expected that the venous pressure would be higher at this time than at any other, except possibly at the peak of the *a* wave, because the A-V valves being closed, blood has been accumulating in the auricles and great veins all through systole. The explanation of this anomaly is thought to be given by consideration of the heart as a pump within a closed system. When arterial blood leaves the chest it lowers the pressure there. This lowering of pressure will be compensated by an inward movement of the chest wall, an inward flow of venous blood, or an upward movement of the diaphragm or, most probably, by all of these acting together. That the chest wall does collapse is

shown by the pneumogram. That venous blood is aspirated into the chest is thought to be shown by the anomalously low venous pressure at the end of systole. This point will be elaborated further in the discussion of the abdominal pneumogram.

*Venous pulse and the abdominal pneumogram.* In figure 3A are shown the venous pulse and abdominal pneumogram. An upward movement in the pneumogram corresponds to a decrease in the size of the abdomen. It will be seen that the general form of the pneumogram is similar to that of the chest. Usually it begins to rise later because the arterial pulse in the abdomen tends to make it expand early in ejection and the collapse is delayed until the venous movement to the chest exceeds the arterial inflow to the abdomen. In this particular case, the arterial pulse causes little or no effect. It is not possible to determine from these records alone how much of the collapse of the abdomen is

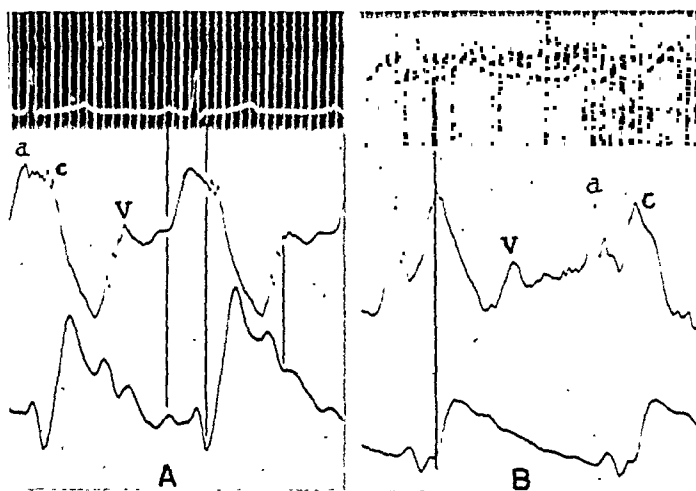


Fig. 3A and 3B. Abdominal pneumograms—lowest curves in records, with venous pulses from the neck. An upward movement in the pneumogram indicates a decrease in volume of the abdomen. Time marks at 0.04 sec. intervals. The subject of figure 3B is the same as 2B.

due to the upward movement of the diaphragm and how much to the movement of venous blood. In either case, however, it is indicated clearly that arterial blood leaving the chest exerts a strong aspirating effect on the abdomen. This same effect must of course be exerted on the veins of the neck. This accounts for low venous pressure at the end of systole. During systole, blood is aspirated from the neck and abdominal veins which process distends the auricles and the veins of the thorax and drains the veins of the abdomen and neck. Following systole, the relaxation of the heart and the decrease of the aspirating action diminish or even reverse the venous inflow so that venous blood accumulates in the neck and the pressure rises to give the upward swing of the *v* wave. This addition to the older views on the venous pulse (Lewis, 1925; Mackenzie, 1925) appears to make its explanation much more satisfactory.

Considering the abdominal pneumogram in more detail, it will be seen that



corresponding to the *a* wave of the neck pulse, there is only a very small downward excursion or expansion of the abdomen. This wave cannot be compared with the venous *a* wave because the recorder is different, but it may be observed that it is small compared with the later abdominal collapse.

In figure 3B, from another subject, a relatively small auricular wave will be seen also. In both 3A and 3B isometric contraction of the ventricles causes rapid expansion of the abdomen which is probably due to the transmission of the apex thrust through the diaphragm. In 3B, a second small expansion is seen just following the first. This is commonly found. It is due to the arterial pulse in the abdomen and it is usually quite small relative to the subsequent collapse.

In figure 3A it will be noted that the initial collapse is followed by a much greater expansion than in 3B. It is not possible to give precise reasons for the variation in wave form because so many factors are involved—arterial flow to and from the abdomen, venous flow to and from the abdomen along with probable movements of the diaphragm. It can be definitely stated, however, that since they both collapse during systole, the chest and abdomen together lose blood to the periphery and regain it during diastole.

Following the second maximum in both 3A and 3B is a phase of rapid expansion occurring at about the same time as the rise of the venous *v* wave. It is concluded that this is due likewise to the relaxation of the ventricles. It occurs later in the abdomen than in the chest by an amount which is similar to that required for transmission to the neck from the chest.

Unlike the neck veins, the abdomen continues to expand after the vibration due to ventricular relaxation rather than to collapse simultaneously with the *v* wave. This may not mean that no additional venous blood moves from the abdomen to the chest during this phase but rather that the diaphragm moves downward to compensate for the movement of the blood.

*The neck pneumogram.* Since the chest and abdomen collapse during systole, the extremities must, of course, expand. In figures 4A and 4B are given two examples of neck pneumograms along with those of the chest. The upward movement in the neck record is due to increase of volume, not decrease as in the chest records. It will be seen in 4A that the neck begins to expand before the chest begins rapidly to collapse. This initial phase of neck expansion is probably due in some way to the apex thrust. The later rapid phase begins with the chest collapse. The rapid descent denoting ventricular relaxation occurs almost simultaneously in the neck and chest records. The deep notch in the neck record just following ventricular relaxation is taken to be the beginning of the venous *v* wave. This can be seen better in figure 4B.

The neck pulse of 4A is largely arterial in form but in 4B, from another subject, the venous pulse waves are more plainly marked. The conditions necessary to record venous waves have not been investigated carefully but it is known that they develop in certain subjects after the breath has been held for some time. In this subject, the apex thrust being ineffective, chest collapse occurs almost simultaneously with the swelling of the neck. The relaxation wave reaches the neck and the chest wall at about the same time. The time required for transmission

along the arteries and veins will depend on the blood pressures and the condition of the vessels, while the time required for the thorax to respond will depend on similar factors involving the elasticity of the lungs, et cetera, consequently, it is not to be expected that there will be uniformity of relationship between the cardiac events as recorded from the neck and from the chest.

*Carotid pulse and thoracic pneumogram.* In figure 5A is shown a carotid pulse almost superimposed on the chest pneumogram which enables their relations clearly to be perceived. In this case there is almost exact simultaneity between the collapse of the chest and the swelling of the carotid, and between the ventricular collapse wave in the chest and the notch in the pulse. These waves have the similarity in form to be expected in a subject in whom the venous return has no sudden variations during the cycle for in such a case the form of the chest movement will be determined largely by the variable arterial flow.

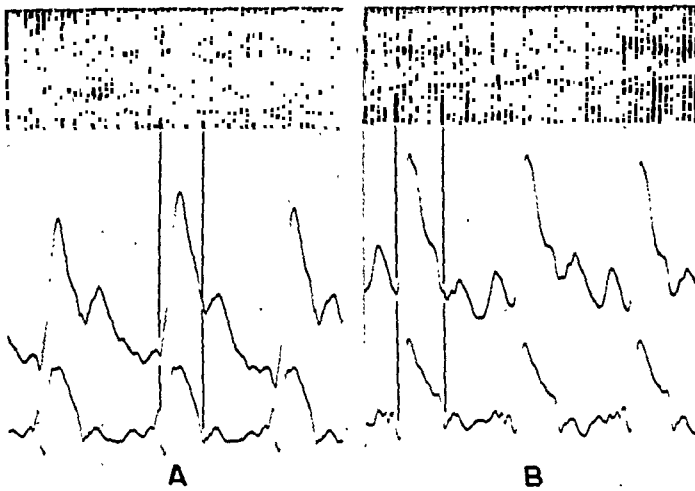


Fig. 4A and 4B. Chest pneumograms—lowest curves, with pneumograms of the neck on two different subjects. An upward movement in the neck record corresponds to an increase in volume; in the chest record it corresponds to a decrease. Time marks at 0.04 sec. intervals. The subject of 4A is the same as 1B and 2A. The subject of 4B is the same as 1A.

In figure 5B, from another subject, it is seen that the collapse of the chest lags behind the swelling of the carotid about 0.02 sec. The early phase of the carotid expansion is probably due to the apex thrust as in figure 4A. The later rapid rise coincides with chest collapse. The end of the collapse wave in the carotid is shown somewhat earlier. This record is from an older and stouter subject than that of 5A. Transmission from the heart to the chest wall may be relatively slower, or the chest wall may have greater inertia. The form of the chest wave differs considerably from that of the pulse. This is probably due to a large inflow of venous blood just following the initial collapse of the chest.

Attribution of the notch in the pneumogram to relaxation of the heart necessitates the same interpretation, in part at least, of the incisura of the arterial pulse. Without discussing in detail all the possible mechanical events associated with semi-lunar valve closure, it appears desirable here to stress the part played by

ventricular relaxation. At the moment that the semi-lunar valves have just closed, the pressures on either side are approximately equal. From this moment on ventricular pressure drops rapidly toward zero, taking away the support on the ventricular face of the valve. Consequently, the relatively high aortic pressure will cause the valve to bulge rapidly and the system, as a whole, to oscillate. The notch in the aortic pulse is probably the primary oscillation of this movement. With the thorax open, ventricular relaxation subsequent to the bulging of the valve may not have much additional effect on the arterial system. With the chest closed, however, it is clear from the evidence above that the effects of mechanical events within the chest are transmitted rapidly throughout the whole trunk. Consequently, the arteries within the trunk are subjected to all these effects. Considering that the mass of the ventricles is several times as great as the total stroke volume of the heart, the results of its movement are

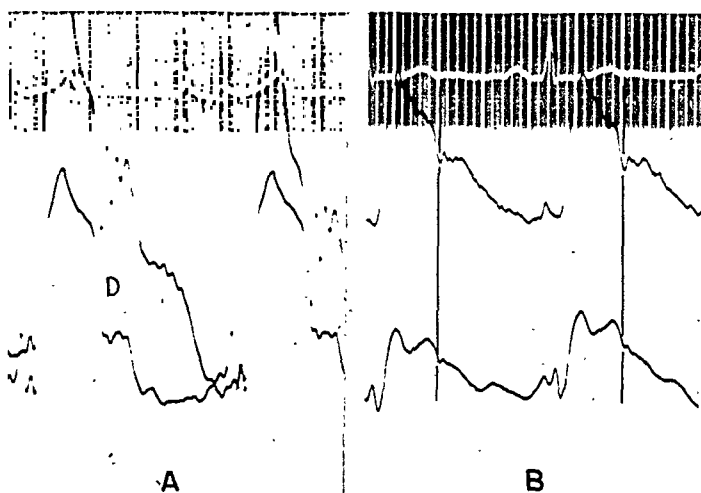


Fig. 5A and 5B. Chest pneumograms of two different subjects—lowest curves, with carotid pulses. Time marks at 0.04 sec. intervals. The subject of figure 5B is the same as 2B and 3B.

likely to be important. For these reasons, ventricular relaxation is thought to be the principal factor in incisura production both through its effect on the aorta by permitting the valve to bulge and through the transmission of its later stages by the closed thoracic system.

*Relation of isometric contraction and chest collapse to Q-R-S.* In figure 6 are plotted the beginning of isometric contraction, the end of the S wave and the beginning of chest collapse for a group of normal subjects, all measured from the beginning of the Q-R-S complex. The beginning of contraction was taken in each case to correspond to the first vibration in the chest pneumogram following the beginning of electrical systole; for example, the one beginning at the right hand timing line in figure 1A. Any lag in the larger waves of the chest pneumogram does not necessarily operate for these small vibrations because they may be transmitted as sound waves through the lungs. There is a possibility that they, in some cases at least, may be due to auricular relaxation, but usually they are

much sharper than anything observable at the time of auricular contraction. Also, in most cases, the beginning of the first sound comes just after these movements while there is very little sound recorded from auricular contraction. Assuming that these vibrations indicate the beginning of the isometric phase, it is found that this phase begins, as will be seen in figure 6, in many subjects at the same time as the beginning of electrical systole. In those cases in which the beginning is apparently late it is possible that the first mechanical movements are not well transmitted. It is concluded, consequently, that mechanical and electrical systole begin very close together.

It will be observed at the top of the figure that the time between the beginning of electrical systole and the collapse of the chest varies in normal subjects from 0.1 to 0.15 sec., the average being 0.126 sec. This time includes isometric contraction along with the time required for arterial blood to begin to leave the chest

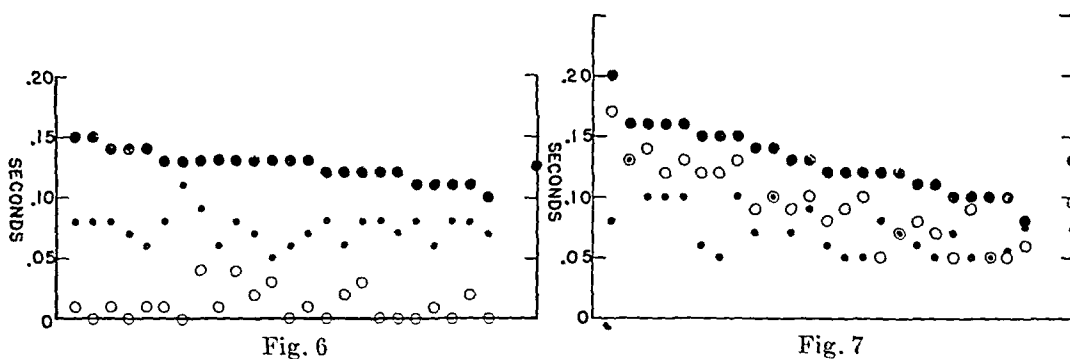


Fig. 6. The beginning of isometric contraction—large circles, end of the Q-R-S complex—small dots, and the beginning of chest collapse—large dots; all measured from the beginning of electrical systole. The three data of each column are from a different subject. The average collapse time is given by the separated dot at the right.

Fig. 7. The end of the T wave—small dots, beginning of isometric relaxation—large circles, end of isometric relaxation—large dots; all measured from the peak of the T wave. The three data from each column are from a different subject. The averages are given by the separated symbols at the right.

and any lag in the chest wall. It has not been possible to ascribe any significance to the differences in normal collapse times except that it is known that the collapse is very early in the fast beating hearts of hyperthyroidism. The duration of Q-R-S does not seem to be a factor in determining collapse time as will be seen from the middle of the diagram.

*Relation of relaxation to the T wave.* In figure 7 the intervals from the peak of the T wave to the end of the T wave and to the beginning and end of relaxation are plotted. It will be observed that there is a wide variation in the interval between the peak of T and relaxation. On the other hand, the interval from the end of T to relaxation is much more constant. In two-thirds of the cases relaxation begins after the end of the T wave, while in one-sixth they begin together and in the remainder relaxation begins before the end of the T wave. These differences may be due in part to the fact that neither the end of the T wave nor the beginning of relaxation is easily determined in some subjects. On the aver-

age, the T wave ends 0.074 sec. beyond its peak while relaxation begins at 0.095 sec. and ends at 0.13 sec.

*Auricular contraction and the chest pneumogram.* Usually the effect of auricular contraction on the chest wall is small but it can be considerable. In figure 8A is shown a chest pneumogram and the venous pulse from the neck. The collapse of the chest occurring with the *a* wave of the venous pulse is almost as large as that due to the ejection of arterial blood. This illustrates in another way that blood migration, in this case venous regurgitation, is the factor which gives rise to the principal volume changes of the chest rather than any direct mechanical action of the ventricular pump itself. The second heart sound occurs in this subject, according to separate determination, just before the first small rebound at the bottom of the first long descent of the pneumogram below the T wave of the

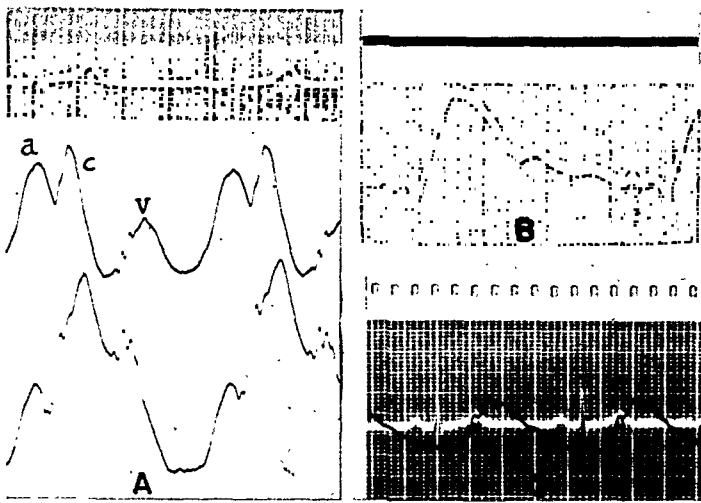


Fig. 8A. Venous pulse—upper record, pneumogram of chest showing large auricular effect—lower record.

Fig. 8B. Pneumogram of chest from subject in sitting position, recorded electrically.

Fig. 8C. Pneumogram of thigh. Upward movement denotes increased volume.

electrocardiogram. The ensuing ventricular relaxation gives rise to further collapse of the chest indicating that temporarily during this phase venous return is reduced to a value below arterial output. This is a clear demonstration that the relaxing heart impedes the venous return in the manner which produces the rise of the *v* wave.

*Chest pneumogram recorded electrically.* In figure 8B is a chest pneumogram taken in the sitting position from the subject of figure 2B by means of the Sanborn heart sound microphone driving the electrocardiograph channel of the Sanborn Stetho-Cardiette. It will be seen that the pneumogram is more regular in outline than in figure 2B. The difference in records from the sitting and the supine positions are much more pronounced in some subjects than in others. They are probably determined by differences in venous blood movements.

*The leg pneumogram.* In figure 8C is a pneumogram from the thigh. The

thigh increases in volume following systole, beginning usually about 0.2 sec. after the beginning of Q-R-S or about 0.07 sec. after the chest starts to collapse. The form of the thigh pneumogram differs considerably in different subjects. Frequently there are several relatively large oscillations on the main wave. The amplitude of the pulse is much smaller than that obtained from the neck with the same apparatus. The neck probably acts as an elastic arterial reservoir to a greater extent than any region of the limb because it supplies the relatively inextensible head.

*The effect of arterial ejection on venous return.* It will be seen that if the chest were rigid and full of fluid the ejection of blood by the heart would have to be accompanied by equal venous return or a vacuum would be created which would make ejection possible only if the force of the pump exceeded atmospheric pressure. Ejection of 60 cc. of blood from an air-filled rigid chest of 3000 cc. capacity, unaccompanied by venous return would create a drop in pressure of one-fiftieth atmosphere or about 15 mm. Hg. This is the maximum possible aspirating force promoting venous return. This maximum will not be reached because the chest is not rigid and because venous blood does return. The actual aspirating pressure is sufficient, as was previously shown (Blair and Wedd, l.c.) to cause about one-half the venous return to occur during ventricular ejection, i.e., in about one-third of the heart cycle. It is indicated by this fact that aspiration of venous blood is an important factor in circulation.

#### SUMMARY

Pneumograms of the chest, the abdomen, the neck and the thigh taken during suspended breathing are related to the electrocardiogram and to venous and arterial pulses. Ejection during systole of arterial blood from the chest creates there a fall in pressure which causes collapse of the chest wall, a probable rise of the diaphragm and aspiration of venous blood from the neck and abdomen. The effect on the abdomen is such that it also collapses. The volumes of the neck and thigh increase to compensate for the decreased volume of the trunk. During diastole, the chest and abdomen expand because venous return exceeds arterial outflow. When decrease of the volume of the chest is recorded in the same direction as increase of pressure in the artery, the pneumocardiogram of the chest is similar in form to a record of the carotid pulse. The pneumogram usually indicates the beginning of isometric contraction of the ventricles, the beginning of ejection from the chest, the beginning and end of isometric relaxation, auricular filling and sometimes auricular contraction. It yields, in general, more information than the pulse while it is much easier to record. It is concluded that the low pressure of the neck veins during ventricular systole is due to the aspirating action of arterial ejection. Aspiration of venous blood is probably an important factor in promoting venous return. The rise of the venous *v* wave is thought to be due to ventricular relaxation. The part played by relaxation of the ventricles in producing characteristic waves in the pneumograms of the chest and abdomen and the notch in the arterial pulse is stressed. The relation of the Q wave of the electrocardiogram to the beginning of ventricular contraction and

of the T wave and the second sound to ventricular relaxation is determined for a group of normal subjects. Electrical and mechanical systole are seen usually to begin very close together.

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# TITRATIONS OF HUMAN SEMINAL FLUID WITH ACIDS AND ALKALIS AND THEIR EFFECTS ON THE SURVIVAL OF SPERM MOTILITY

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Many problems dealing with fertility, artificial insemination and contraception involve a knowledge of the environmental conditions which affect the survival of human spermatozoa. It has been known for some time that among these conditions, the pH of the surrounding medium is an important factor which requires further study. Muchat (1) indicated that the optimum pH for motility of human spermatozoa lies between pH 8.5 and 9.5 and that motility stops when the pH is less than 6 or greater than 10. Voge (2) reported spermicidal tests with human semen and lactic acid. He noted that a pH of 4.9 to 6.4 arrests sperm motility and that below pH 3.5 revival by neutralization is not possible. All his pH measurements were carried out colorimetrically which may involve some ambiguities. Baker (3a) reports that all cavy spermatozoa are immobilized in thirty minutes by solutions of sulfuric, acetic, tartaric and citric acid when the pH of the medium is below 5. Since the specimens in his tests differ in both the medium and species from human semen, direct comparisons with human spermatozoa cannot be made. Shedlovsky (4, 5) summarizes the meager available information on the spermicidal action of acids as dependent on the pH and buffer capacity of the fluids involved.

The maintenance of a favorable pH or the attainment of one which is lethal to spermatozoa will depend on the buffer capacity of seminal fluid. The only quantitative data which we found in this connection are those of Baker (3b, 3c), Baker, Ranson and Tynen (6) and Tynen (7) who established several points on the titration curve, with hydrochloric acid but only down to pH 6.0, and Schersten (8) who made two determinations to a pH of 4.9. Our report covers a much wider range of pH since we were also interested in spermicidal effects.

It has been our object to make a more precise and complete study of the buffer capacity of semen and the effect of changing the pH of the medium on the survival of the motility of spermatozoa.

**EXPERIMENTAL.** The semen specimens were obtained in glass vials from 12 donors. Precautions were taken to avoid using a given donor more than once a week. Spermicidal tests were begun on individual specimens within two hours from the time they were obtained and carried on for several hours. The age of the specimen affects the spermicidal tests sufficiently to require that a series of such tests should be started within an interval of about one hour in order to have the results of a particular series on a comparable basis (9a).

<sup>1</sup> This work was made possible by a grant from the National Committee on Maternal Health.



A series of solutions of hydrochloric, lactic, citric, tartaric, maleic, phosphoric, acetic and monochloroacetic acid and sodium and potassium hydroxide and ammonia of known concentrations was prepared by the dilution of standardized stock solutions. In order to distinguish between the spermicidal effects due to the pH and those due to changes in salt concentration, all the stock solutions and dilutions made from them contained 0.9 gram per 100 cc. (0.154 M) NaCl except for KOH which contained 1.15 grams per 100 cc. (0.154 M) KCl.

Equal volumes of either 0.10 or 0.15 cc. of semen and acid or alkali solution were measured out from 0.5 or 1.0 cc. tuberculin syringes and mixed thoroughly by drawing the mixture into the syringe and expelling it into a 3 cc. vial ten times. The use of such small amounts permitted many tests to be made with each specimen. A small drop of the mixed fluids was placed upon a slide, a cover slip placed on it and the preparation examined by means of dark field illumination. Observations were made with both low and high power magnification until every spermatozoön had become immotile, so that both translational as well as vibrational motion was absent. The number of minutes taken for this to occur from the time mixing was started was defined as the spermicidal time.

As a control test, equal volumes of semen and 0.14 molar (2.8 per cent) aqueous solution of potassium acid phthalate (initial pH 4.0) were mixed (9b). The pH of such mixtures for various specimens ranged from 5.0 to 5.4. A specimen was not suitable for comparative spermicidal tests when the spermicidal time for such a mixture was less than twenty minutes. These tests were performed at room temperatures (20–26°C.).

The pH measurements were made with a shielded glass electrode assembly of the type described by MacInnes and Longworth (10) using a Compton Electrometer and a L. and N. Student Type Potentiometer. The condenser shaped electrode had a capacity of 0.25 cc., and it was possible to make measurements with less than this volume. The introduction of the mixture into the electrode was facilitated by having the top of the latter ground to fit the standard tip of the syringes. It was possible to make these measurements with a precision of  $\pm 0.01$  pH unit.

As a standard of pH we have adopted 0.05 molar potassium acid phthalate solution, which has a pH of 4.00 at 25°C. To insure the correct performance of the electrode, it was always checked with another known phosphate buffer (pH 7.73) and 0.1 N HCl (pH 1.08) before and during the experiments. After a considerable number of determinations, there was a tendency for the electrode to give values too low by 0.1 to 0.2 pH unit, but this error due perhaps to protein films was obviated by washing the electrode immediately after each determination with a little dilute HCl solution (0.1 N), and following with a considerable amount of water. If the latter is forced by a 20 cc. syringe through the electrode at high velocity, it tends to remove films.

The carbon dioxide content of the semen was determined by the Van Slyke volumetric method (11).

**RESULTS.** The titration curves with HCl and NaOH solutions are given in fig-

ures 1 and 2 respectively. In these curves the pH of the mixtures of acid or alkali with semen are shown as a function of the milliequivalents of acid or alkali added per cubic centimeter of a mixture of equal volumes of semen and acid or alkali. The upper curves (fig. 1) lying fairly close together, indicate what may be expected for the normal run of semen specimens mixed with acid, an occasional curve lying at some distance below the others shows the occurrence of a relatively poorly buffered specimen. Eleven additional titrations of semen with HCl were made besides those given in figure 1. In these determinations mixtures of equal volumes of semen and 0.50 N HCl gave a pH range from 0.97 to 1.24 with an average value of 1.1.

The titration curves for the other acids which are not shown gave higher pH values than for the corresponding points on the HCl curves, and a specimen which was poorly buffered against one acid was found to act similarly with the other acids.

Figure 3 summarizes the data for the pH of equal volumes of semen and solutions against the spermicidal time for all the acids tested. Region A covers the range of the results for monochloroacetic and acetic acid and region B covers the range of the results for hydrochloric, lactic, tartaric, citric, phosphoric and maleic acid. In each group all the experimental points are entirely in the region indicated, although on the average the spread of values is only about half as great as that shown.

Figure 4 shows typical curves of pH against spermicidal time for tartaric, phosphoric, maleic, citric and monochloroacetic acid, for a single semen specimen. In figure 5, for two other semen specimens marked 1 and 2, the difference is demonstrated between monochloroacetic and acetic acid against citric and tartaric acid which in general have effects similar to hydrochloric and phosphoric acid.

In figures 3 to 5 it may be noted that spermicidal times above 120 minutes are not indicated. When the time for complete immobilization of spermatozoa is much greater than this, the values cannot be quantitatively compared with shorter periods of time.

In order to give an example demonstrating quantitatively the differences between acidic solutions of different buffer capacities, we compared solutions of mixtures of lactic acid and sodium lactate and tartaric acid and sodium tartrate with solutions of the corresponding acids. For the buffer solutions, the total concentration of anion constituent was 0.5 normal in each case. No sodium chloride was used in these experiments. To indicate how much less such buffer solutions increase in pH than solutions of the acids alone when mixed with semen, the change for one solution will be given. When a tartaric acid solution with a pH 2.5 is mixed with an equal volume of semen, the pH will be 6.7, whereas for the tartrate buffer it will be 3.5. In each case the spermicidal time is determined by the pH of the mixture so that for pH 6.7 it would be more than 300 minutes and for pH 3.5 about 1 minute for the same specimen of semen.

The characteristic spermicidal effects of alkalis are shown in figure 6 for a particular semen specimen. NaOH and KOH have essentially the same effects, but ammonia solutions show a specific effect apart from the pH.

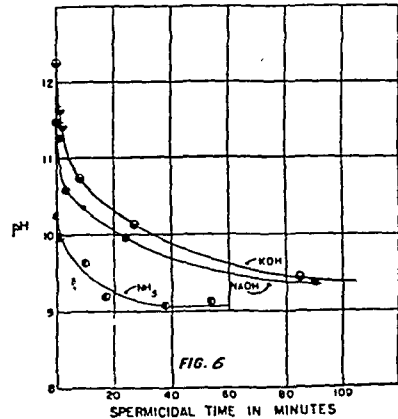
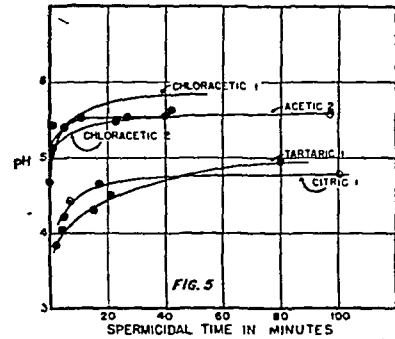
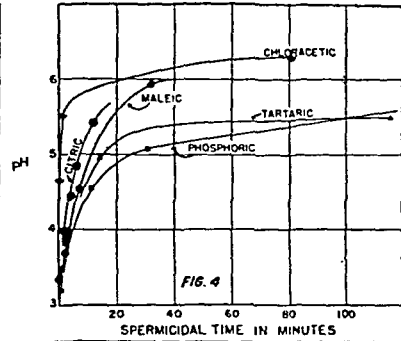
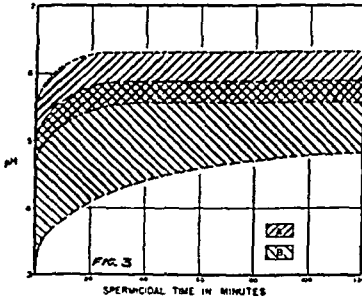
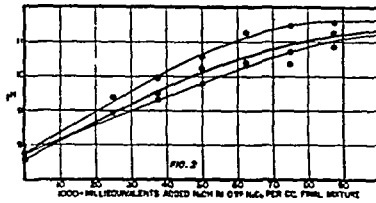
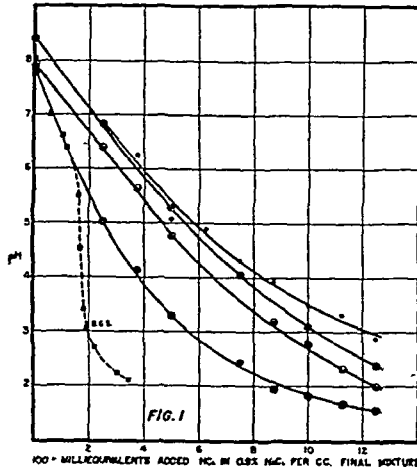


Fig. 1. Titration of semen with HCl solution. pH against milliequivalents added acid per cubic centimeter of final mixture of equal volumes of semen and acid solution. Each curve represents a different semen specimen. Curve marked *B.G.S.* represents titration for Baker's Buffered Glucose Solution (3b).

Fig. 2. Titration of semen with NaOH. pH against milliequivalents added base per cubic centimeter of final mixture of equal volumes of semen and alkali. Each curve represents a different semen specimen.

Fig. 3. Summary of data for acids: pH of mixture of semen and acid solutions against spermicidal time. Region (A) monochloroacetic and acetic acid, region (B) hydrochloric, lactic, tartaric, citric, phosphoric and maleic acid. Points for all tests made always fall in the region indicated.

Fig. 4. pH of mixture of semen and acid solutions against spermicidal time for phosphoric, tartaric, citric, maleic and chloroacetic acid. All curves for the same semen specimen.

Fig. 5. pH of mixture of semen and acid solutions against spermicidal time for citric, tartaric, acetic and monochloroacetic acid. 1 indicates one semen specimen. 2 indicates another semen specimen.

Fig. 6. pH of mixture of semen and alkali solutions against spermicidal time for NaOH, KOH and NH<sub>3</sub>. All curves for the same semen specimen.

It has been noted that semen on standing, first becomes more alkaline and our data indicate that this change is most likely due to loss of carbon dioxide. Our measurements of the pH of semen gave a range from 7.6 to 8.4 for various specimens, but in most cases the values were less than 8.0. The result of the experiment given in table 1 shows how the carbon dioxide content affects the pH of the semen. Ageing has been shown by others first to make semen more alkaline and then more acid, the latter effect has been attributed to the formation of more lactic and other acids. The formation of acid was avoided in our experiments (table 1) by the addition of phenyl mercuric nitrate at the start.

DISCUSSION. The pH of seminal fluid as reported by a number of investigators covers a range from pH 7.0 to 8.9 and unless precautions are taken to prevent loss of carbon dioxide the pH increases. We have shown that it is most likely that the higher values were for semen that had lost carbon dioxide on ageing.

TABLE 1

*Change in CO<sub>2</sub> content and corresponding pH of semen*

(a) Specimen kept in jar with cover on loosely. Phenyl mercuric nitrate added at start of experiment. All sperms immotile.

AGE IN MINUTES FROM TIME COLLECTED	MILLIMOLES CO <sub>2</sub> PER LITER	PER CENT CHANGE IN CO <sub>2</sub> FROM INITIAL VALUE	pH	CHANGE IN pH
8	21.3		7.73	
243	11.1	-48	7.78	+0.05

(b) Composite of two semen specimens with phenyl mercuric nitrate added at time of collection. Temperature 22°C.

TREATMENT	MILLIMOLES CO <sub>2</sub> PER LITER	PER CENT CHANGE IN CO <sub>2</sub> FROM INITIAL VALUE	pH	CHANGE IN pH
Mixture 2 hours old. Kept in covered container. ....	24.8		7.57	
Mixture kept 6 hours over concentrated KOH solution. ....	6.7	-73	7.89	+0.32
Mixture kept 6 hours under 1 atmosphere pressure of CO <sub>2</sub> . ....	81.9	+230	6.43	-1.14

In our opinion, the buffer capacity of the semen is far more important than its pH and this value gives no clue to the buffer capacity as can be seen from figure 1. The reports of Baker, Ranson and Tynen (6) give the amounts of 0.01 N HCl necessary to bring the pH of 1 cc. of semen to 7.1, 6.7, and 6.0. Their range of values of the amount of acid added agree well with the corresponding points on our curves (fig. 1) but do not cover an adequate range of pH to indicate its buffer capacity to larger quantities of acid. Schersten (8) reports two titrations of semen with HCl solution using the quinhydrone electrode. He took 1 cc. of a human semen specimen and added 0.1 cc. increments of 0.1 N HCl up to 1.0 cc. and determined the pH. For the last point of 0.10 milliequivalents of HCl per cc. of semen, Schersten obtains a pH of 4.85 which corresponds to our pH values for this point which ranged from 4.8 to 5.4 for different specimens (fig. 1).

It may be of interest to examine the buffer capacity of two artificial fluids which have been used as diluents for semen or suspending media for spermatozoa. One solution of this kind is that of Baker (3b) known as B.G.S. (buffered glucose saline) which has the following composition:  $\text{KH}_2\text{PO}_4$  0.1 gram,  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  3.0 grams (equivalent to 6.0 grams  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ )  $\text{NaCl}$  2.0 grams, glucose 30.0 grams and water to make 1000 cc. The pH of this solution is 8.1 and our titration curve with hydrochloric acid (fig. 1) resembles that of the lowest curve for semen over a small range to pH 6.0, but at lower pH values, it is poorly buffered in comparison even with the least buffered semen specimen tested. Another solution proposed by Baker (3b) is a mixture of 1.4 cc. of 6 per cent  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  with 1.6 cc. of egg white. This solution compares very favorably with semen as an alkaline buffer but the use of various samples of egg white show considerable variations.

From an examination of the data as well as from spermicidal tests with other substances, it becomes apparent that quantitative comparisons of spermicidal tests can be made only for a particular semen specimen. The most important reasons for this are variations in both the seminal fluid as well as the spermatozoa. For example, the titrations in figure 1 give a range of values for different specimens although each specimen gives a reasonably smooth curve.

For hydrochloric, lactic, citric, tartaric, maleic and phosphoric acid, the spermicidal properties are primarily the same function of the pH of the medium and no significant specific effects are indicated.

Climenko (12) suggests that maleic acid has spermicidal properties which "seem to depend on the fact that it inhibits the oxygen consumption of the cell." His tests were made in buffered glucose saline solution with cavy spermatozoa, but apparently the acidic properties of the maleic acid were not considered a significant factor which determines its spermicidal properties. Our results show that maleic acid has spermicidal properties with human spermatozoa which are comparable to those of citric and tartaric acid and that no marked specific effect is indicated.

Osterhout (13) points out that plant cell membranes are particularly permeable to undissociated molecules. If this argument is applicable to sperm cell membranes, it may lead to a partial explanation of the specific effect of acetic acid and ammonia when compared with stronger acids and alkalis respectively. Monochloroacetic acid has a larger dissociation constant than lactic acid, but shows specific spermicidal properties when compared with it. In this case, the specific effect may be related to the inhibition of the glycolysis of spermatozoa comparable to that shown for iodoacetic acid.

#### SUMMARY

Methods are described for carrying out titrations of semen with acids and alkalis using a glass electrode assembly suitable for volumes as low as 0.15 cc. with a precision of  $\pm 0.01$  pH and for the determination of the relative spermicidal properties of such mixtures.

Some comments and measurements are made on the relative buffer capacity of solutions proposed as diluting media for semen.

Changes in the pH of semen as a function of the CO<sub>2</sub> content are shown by keeping the semen over concentrated KOH solution or under one atmosphere pressure of CO<sub>2</sub>. The pH varies from 7.9 for 6.7 millimoles CO<sub>2</sub> per liter to 6.4 for 81.9 millimoles CO<sub>2</sub> per liter.

Titration curves and the survival time of the motility of spermatozoa for semen with hydrochloric, lactic, citric, tartaric, maleic, phosphoric, acetic and monochloroacetic acid, sodium and potassium hydroxide and ammonia solutions are reported. For the first six acids, each dissolved in solutions containing 0.9 gram NaCl per 100 cc. and mixed with equal volumes of semen, the time for complete immobilization of the spermatozoa was less than half a minute in every case tested if the pH of the mixture was less than 3.0. Acetic and monochloroacetic acid show specific spermicidal effects. For these acids, the time for the complete immobilization of spermatozoa is always less than half a minute if the pH of the mixture is less than 4.7.

As an example of the above effects, the marked superiority of the spermicidal properties of well buffered tartrate and lactate solutions over solutions of the corresponding acids alone is shown on a quantitative basis.

Sodium and potassium hydroxides give a time for the complete immobilization of spermatozoa of less than half a minute when the pH of the mixture is greater than 12.2, whereas in the case of ammonia specific effects are indicated so that the same spermicidal properties are obtained when the pH is 10.3 or higher.

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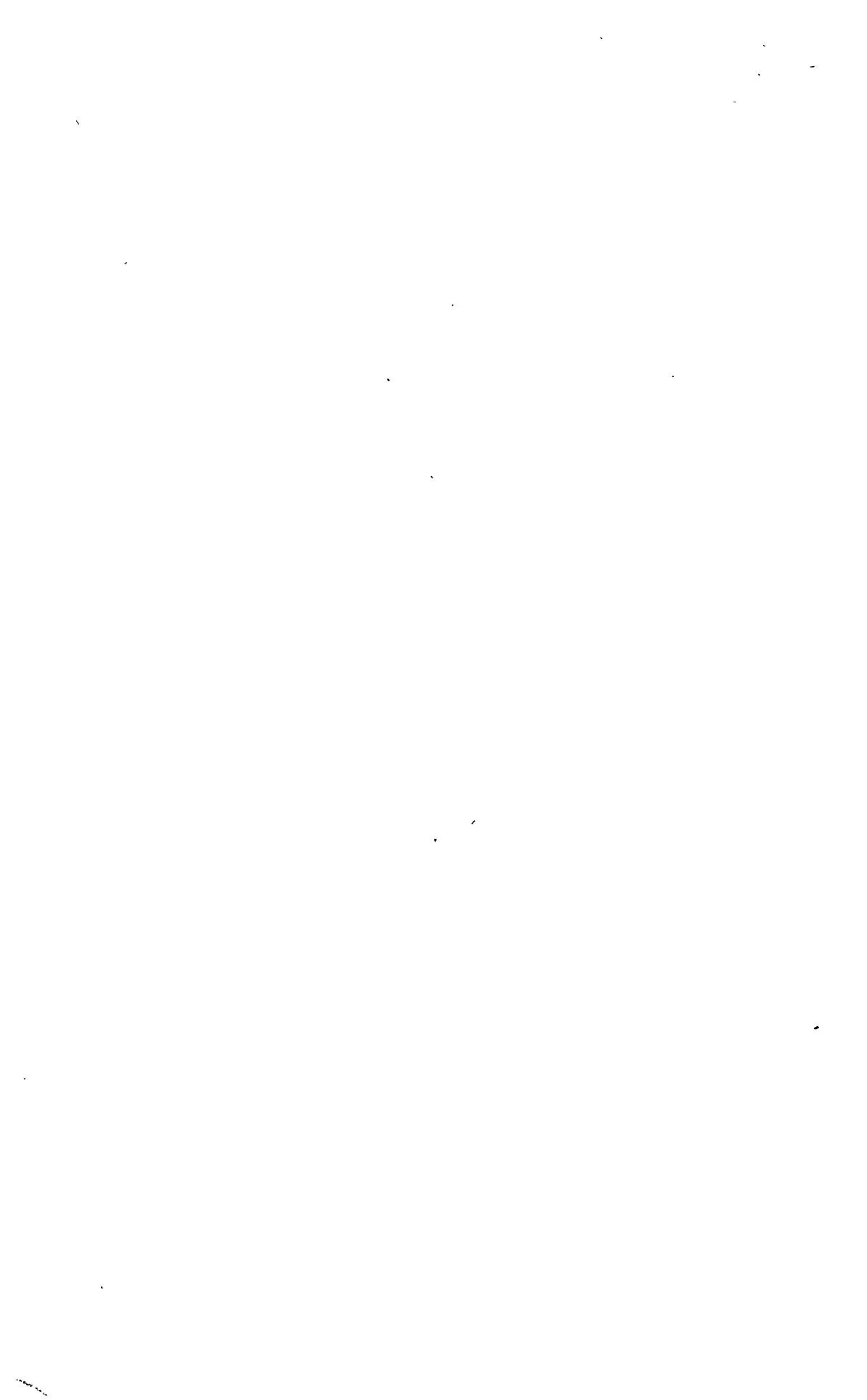


#### ERRATUM

The authors of the paper "Observations on the Accuracy of the Thermostromuhr" beginning on page 250 of no. 2 of this volume (136) desire to record the following corrections:

- p. 256. Delete sentence beginning line 9 and substitute: "In the right coronary flow is frequently always forward but the appearance of back flow components is not uncommon."
- In line 13 after the words "can appear" insert reference (5).
- p. 261. Under "References" correct to read as follows:
- (2) Gregg, D. E., A. Rotta, R. W. Eckstein, R. E. Shipley and J. T. Wearn. *Proc. Soc. Exper. Biol. and Med.* 49: 267, 1942.
  - (4) Eckstein, R. W., D. Book and D. E. Gregg. *This Journal* 135: 772, 1942.
  - (5) Pritchard, W. H., A. S. Weisberger, E. F. Schroeder, R. E. Shipley and D. E. Gregg. To be published.





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## THE ACTIVITY OF THE PACEMAKER PREVIOUS TO THE DISCHARGE OF A MUSCULAR IMPULSE<sup>1</sup>

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In the present paper it will be shown for the ureter and the intestine that a characteristic weak activity always precedes, and apparently initiates, the spontaneous discharge of an all or none conducted impulse. This non-conducted activity consists either in a gradually increasing negativity or in rhythmic potential variations. In the ureter, furthermore, a slight rise in tonus, similar in its time relations to the electric change, precedes each spontaneous contraction.

The initiation of spontaneous activity in muscle was previously studied only in the heart. Adrian (1) and Eccles and Hoff (16) have failed to detect electric changes preceding the discharge of an impulse in the pacemaker of the vertebrate heart. In the heart of a snail Arvanitaki (4), however, clearly demonstrated slow changes in electric potential and in tonus previous to each beat. His results agree in their fundamental aspects with my own observations on vertebrate smooth muscle. Recently, similar results were also obtained for vertebrate cardiac muscle (15). The similarity of the phenomena in these histologically so different muscles derived from different kinds of organisms is significant because it suggests that the processes underlying automaticity are essentially the same in all muscles.

The artificial initiation of rhythmic activity in skeletal muscle by chemical substances and by constant electric current closely resembles normal automaticity. It is interesting, therefore, that the discharge of impulses in these tissues (for skeletal muscle cf. 2, 5; for nerve cf. 4) is initiated by local potentials which are similar to those which will be described here for the normal activity of smooth muscle and which have also been found in vertebrate cardiac muscle (15). Recent studies, furthermore, have shown that a local reaction also precedes the discharge of an impulse produced by electric shocks and by motor nerve impulses. It appears probable, therefore, that all conducted responses of nerve and muscle, whether spontaneous or elicited by stimuli are initiated by

<sup>1</sup> Aided by a grant from the American Academy of Arts and Sciences.

local graded reactions which are characterized electrically by a gradual rise in negativity.

**METHODS.** The isolated ureter of the dog, cat and guinea pig, and the small intestine of the guinea pig were used. The ureter was dissected up to the renal pelvis and carefully freed of extraneous tissue.

In the ureter sometimes spontaneous activity did not begin until several hours after the preparation was set up and then contractions occurred infrequently. Asphyxia during the dissection seems to have been the chief cause of this difficulty. Rapid dissection and the use of chilled and aerated Ringer's solution improved the results. Furthermore, it was found that motility was less in Ringer's solution made up in copper distilled water than in solutions prepared with glass distilled water. The exact composition of the Ringer's solution seems to be of less importance. Preliminary experiments showed that the action of calcium and potassium ions on the ureter is essentially like that on the heart. A moderate increase in calcium increases the duration and strength of the contraction without appreciably influencing the rate; increase in potassium has the opposite effect. The pH has no appreciable effect within a wide range. Ringer-Locke solution buffered with phosphate was found nearly optimal for the experiments.

The technique of recording potentials was the same as that described in the following paper. Because the changes studied were slow, a mechanical recorder writing on a smoked drum was ordinarily used (natural frequency 40 per sec.). A comparison of the graphs with those obtained with an oscillograph showed that no distortion was introduced by the recorder. It should be noted, however, that the records do not correctly indicate the amplitude of the conducted responses because the latter are usually more than fifty times stronger than the local activity and, consequently, are largely cut off by the characteristics of the amplifier.

The results which will be reported here show that local potentials may occur simultaneously over a wide region of a muscle. Since the records merely indicate the difference in the activity at the two leads these potentials may escape attention if both leads are on an active region. To avoid this possibility it would be desirable to place one lead on an injured region so as to obtain monophasic potentials. However, serious difficulties are encountered in recording monophasic potentials in this manner for more than a few minutes (cf. 14) and, furthermore, complications are introduced by the injury itself because the border of the injured region may temporarily act as a pacemaker. The conditions under which these difficulties were avoided will be described later.

In the ureter the origin of the impulses was determined by placing the leads close together and observing which of the leads became negative first. This procedure was repeated, shifting the leads in the direction from which the impulses were coming. At the origin of the impulses the diphasic action potentials begin to become inverted.

In one series of experiments the contractions of the ureter were recorded by a very sensitive isometric lever. The preparations were immersed in Ringer's solution.

**RESULTS.** *Ureter.* This organ is unusually favorable for the study of the activity of the pacemaker because normally discharges occur at rather long intervals and originate in a distinct region, the extreme renal end. There is a gradient of diminishing automaticity away from this region as indicated by the fact that after cutting the organ the caudal part discharges at a slower rate than the renal region. The middle region alone, if suspended in air, discharges only rarely.

At the beginning of an experiment, as soon as spontaneous activity begins, the

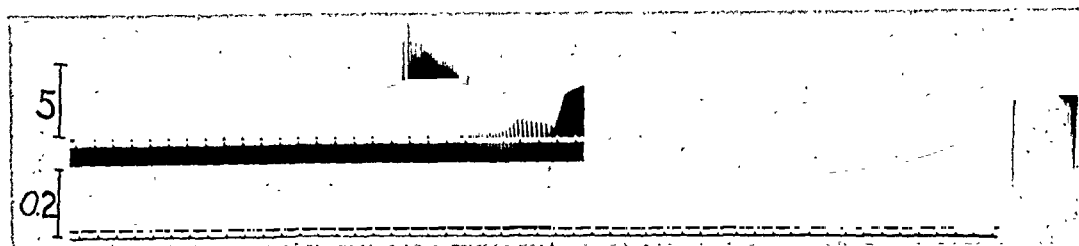


Fig. 1. Slow rise in negativity of the pacemaker preceding a spontaneous peristaltic wave in the ureter of the guinea pig, recorded by oscillograph. First lead on pacemaker, second 2.4 cm. distant. Both records from the same preparation at different amplification. On left calibration in mVolt. Temp. 36°. Time  $\frac{1}{5}$  sec.

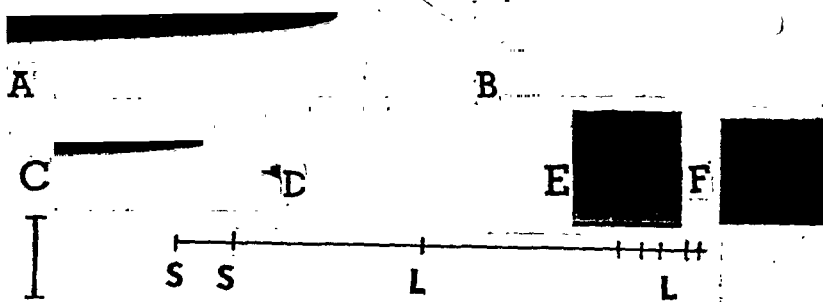


Fig. 2. Records showing magnitude of local potentials at different distances from pacemaker of the cat's ureter. First lead: in A on pacemaker, in B 3 mm., in C 5 mm., in D 7 mm., in E 1 cm. closer to second lead. F: contraction produced by electric shock and conducted antidromically, first downstroke shock artifact. Below: diagram of arrangement of electrodes. S: stimulating electrodes. L: leads to amplifier. Calibration: one mVolt. Temp. 36°. Time, seconds.

origin of the impulses was determined as described above. It was usually found near the renal end of the organ. If one lead was placed on the renal end, the other 2 or more cm. caudad, every peristaltic contraction was preceded by slow potential changes which occur in two forms: 1, a gradually increasing negativity of the renal end, the rise becoming progressively faster until the moment of discharge of the conducted response (fig. 1, 2); 2, potential oscillations which gradually increase in magnitude until a discharge occurs (fig. 3). If rhythmic potentials are present, the impulses are discharged near a crest and at a time when the renal end is negative.

It can be shown that the potentials preceding a spontaneous discharge are produced in the region where the conducted responses originate, usually at the renal end of the ureter. They are largest if one lead is near the renal end of the ureter, they diminish rapidly caudad and are practically absent if the lead is more than 1 cm. from the renal end of the organ (fig. 2). A change in the position of the other lead has no influence on the magnitude of the slow potentials provided it is at least 2 cm. away from the renal end. In this connection it should be remembered that electronic spread of potentials is insignificant in smooth muscle. The distribution of the potential variations near the pacemaker, therefore, correctly represents the processes in the region of the leads.

In exceptional cases impulses also have originated in the middle or caudal region of the ureter but usually only for short periods of time. In this case

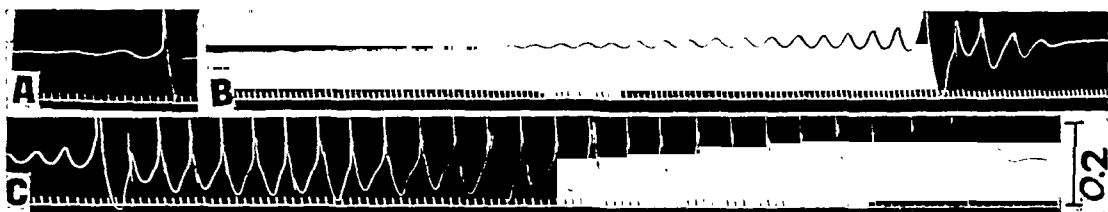


Fig. 3. Oscillatory local potentials from ureter of guinea pig. First lead on pacemaker. A: single discharge. B: long series of oscillations in depressed preparation. C: a group of discharges. Calibration: 0.2 mV. Temp. 34°. Time, seconds.



Fig. 4. Effect of premature contractions on rhythmicity of the cat's ureter. First lead on pacemaker. Two premature peristaltic waves, produced by electric shock and conducted antidromically. Arrangement of electrodes as in figure 2. Downstroke preceding premature contraction shock artifact. Calibration: 1 mV. Temp. 36°. Time, seconds.

the characteristic local potentials were observed near the place of origin, whereas the renal end appeared silent.

The observations just mentioned show that the slow potentials have nothing to do with the conduction of impulses. This is also demonstrated by the fact that slow potentials are absent in responses induced by an electric shock (fig. 2F, 4). It can be concluded, therefore, that these potentials are the expression of a non-conducted activity of the pacemaker preceding the discharge of impulse. They may be interpreted as a slow depolarization of the cell surface.

The magnitude of the slow potentials up to the moment when the discharge occurs is usually only about one-fiftieth of the magnitude of the conducted response. The possibility that these weak electric changes are artifacts is improbable because in preparations mounted in a moist chamber only drastic movements produce detectable potential changes and because the local potentials are not accompanied by visible movements.

To determine whether the local potentials are accompanied by a contraction, the tension of the muscle was recorded. A short piece, about 1 cm. long, from

the renal portion of the dog's ureter was used. Previous to each spontaneous contraction there was a gradual rise in tonus or a brief series of tonus waves (fig. 5). In agreement with the small size of the local potentials the mechanical changes were very weak. The tension developed in the ureter of a dog of moderate to large size never exceeded 40 mgm., only about one-fiftieth of the tension produced in a peristaltic contraction. The rhythmic variations in tonus were not observed as clearly as the corresponding potential changes, partly because of the smaller sensitivity of the tension lever as compared with the electric recording system; partly also, perhaps, because of some incoördination between different parts of the preparation which tends to smooth out the oscillations.

The magnitude of the tonus changes varied greatly under different conditions. It was greatest if the organ showed great motility. In muscles where spontaneous beats occurred at very long and irregular intervals sometimes the increase in tonus before a contraction was not detectable. Such cases can be explained by assuming that a very small portion of the muscle was involved in the initiation of a contraction. It seems probable that the depolarization

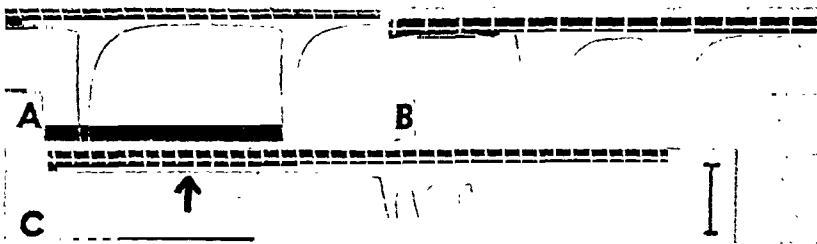


Fig. 5. Tension records from ureter of dog showing gradual rise in tonus (A) and tonus waves (B) before a spontaneous contraction. Preparation 1 cm. long, taken from the renal region. C, taken before spontaneous activity started, shows rise in tonus after adding 1 drop of adrenaline 1:1000 to bath (indicated by arrow). Calibration: 0.2 gram. Temp. 37°. Time, 5 sec.

of a large region, as normally occurs, is unnecessary for the initiation of an impulse. Occasional negative findings, therefore, do not invalidate the positive results obtained in the more active preparations.

A tonus increase also invariably precedes the response elicited by adrenaline (fig. 5C) and by stretching (15).

*Small intestine of the guinea pig.* The study of the initiation of spontaneous contractions in the intestine presents much greater difficulties than in the ureter because bursts of impulses may be discharged simultaneously from many regions of the organ and because the origin of the discharge varies almost continuously. Unfortunately, there is no entirely satisfactory method of recording simultaneously local potentials and conducted impulses. If the leads are widely separated, as was the case in most of the work of previous investigators, the action potentials of conducted impulses are greatly distorted and difficult to recognize (cf. 11). If the leads are close together, as in my earlier studies (11), the discharge of impulses can be recorded clearly, but local potentials which involve appreciable parts of the muscle are masked.

However, an approach to a monophasic potential without mechanical injury

was obtained by drawing one end of the preparation into a narrow glass tube and attaching one lead to this part of the muscle. The region inside the tube became asphyxiated and inactive. The inactivity under one lead greatly simplified the records. Purely monophasic potentials were usually not obtained, probably because the active lead was too far removed from the inactive region (cf. 14).

The records obtained under the conditions just described show two distinct types of potential changes. While the muscle appears quiescent there are fairly regular smooth rhythmic potential variations at a frequency of about one every 1.2 seconds. In addition there are bursts of impulses which accompany the peristaltic waves and have previously been described (11). Usually the peristaltic waves start out from the same region repeatedly. If the active lead happens to be on such a region the impulses rise from the negative crest of some of the

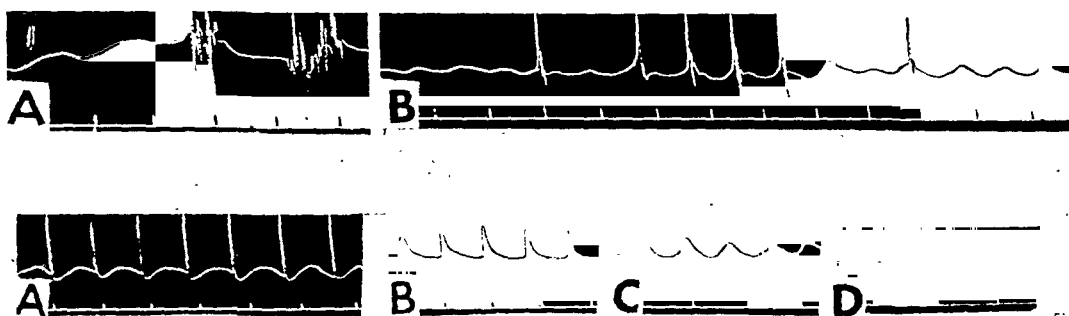


Fig. 6. (top) Potentials from the small intestine of the guinea pig showing local potentials and impulses. One lead on part of the muscle inside glass tube. A: fresh preparation, peristalsis. B: obtained about 30 minutes after A. Temp. 34°. Time, seconds.

Fig. 7. (bottom) Potentials from a contraction ring of the small intestine of the guinea pig. Preparation was quiescent except for slight contraction waves in contracted ring. A: active lead in center of ring, B: 2 mm., C: 4 mm., from center. D: lead outside ring. Temp. 34°. Time, seconds.

slow waves. A shift of the pacemaker occurred frequently and was often indicated in the potential record (fig. 6A).

The relation between the slow potentials and the discharge of impulses could be observed more clearly one half to one hour after the preparations had been brought into the moist chamber, and after they had become less active. Their activity then resembled pendular movements. Single impulses or small bursts of impulses were discharged at a fairly regular frequency. These impulses also arose from the negative crest of the potential waves (fig. 6B).

Shortly before the activity of a preparation ceased permanently, contraction rings often remained at the same place for long periods of time while movements were slight or absent. Strong rhythmic potential variations occurred in this region. At the place of strongest contraction impulses were discharged (fig. 7). On both sides there was a region where the impulses were very weak and still farther away there were only slow potential waves without any discharge of impulses. Such slow waves, however weaker and less regular, were also found

on the rest of the intestine which appeared inactive. It seems that impulses were discharged from a point in the central part of the contraction ring and that they were conducted decrementally in both directions for a few millimeters.

The slow potential variations just described probably are identical with those which have been recorded by previous observers in the intestine of the rabbit (3, 8, 9, 10) and dog (17). They have been demonstrated most convincingly by Berkson who observed that they continue almost unchanged after movements have been stopped by adrenaline, atropine and other drugs. I have confirmed this result for the intestine of the guinea pig. Balassa (6) reported a similar observation for the uterus of the cat. As pointed out by Berkson (9), these facts show clearly that the slow potential waves are not the action potentials of the rhythmic contractions of the intestine. That these contractions are due to the discharge of conducted impulses is shown by the fact that they are accompanied by a regular series of spike potentials (11).

An explanation for the slow potentials is suggested by the observation that each discharge is preceded by a phase of rising negativity. It appears, therefore, that the slow potentials are analogous to those of the other muscles studied and represent local potentials which initiate impulses. These potentials can be regarded as an expression of the automaticity of the muscle. Whether they actually produce impulses naturally depends also on whether muscular excitability is high enough for the conduction of impulses. It appears plausible, therefore, that the slow potentials may continue while no contractions occur, as is the case under the influence of adrenaline and as it has also been observed in the ureter and in cardiac muscle (15) during a state of low excitability. Berkson's suggestion that the slow potentials are a regulating influence on the muscular rhythm, although not originating it, agrees in part with the explanation offered here, but his assumption that the potentials are caused by the action of the intrinsic nervous plexus appears unnecessary.

The action of some of the other drugs studied might seem to contradict the explanation offered here. Nicotine and curare were found to diminish or abolish the local potentials while the contractions became less frequent and often stronger (10). This result can be explained by assuming that the drugs diminish the tendency of the cell surface to depolarize spontaneously. In this case it is possible that the local potentials originating the contractions are produced only in small regions of the muscle and, therefore, appear smaller or may escape attention.

DISCUSSION. 1. *The nature of the local potentials and their relation to automaticity.* The observations reported here suggest that a spontaneous, non-conducted activity, indicated by a change in electric potential and of tonus, is the basis of the automaticity of visceral muscles. The local potentials differ from impulses not only by the absence of conduction but also by their slowness. The assumption that the underlying process is an asynchronous discharge of impulses conducted for short distances is unlikely in view of the smoothness and regularity of the process. The slow potentials, therefore, can best be interpreted as a non-conducted depolarization of the cell surface. This interpretation



agrees with that which has been given to similar potentials in nerve fibers and skeletal muscle during the artificial initiation of impulses by chemical substances.

The tendency of the cell surface to depolarize spontaneously is not restricted to the exact region where the impulses originate. Thus, in the intestine, the local potentials can be found in all regions of the muscle, in agreement with the fact that the degree of automaticity is fairly uniform throughout the intestine. In the ureter the local potentials can usually be recorded from a region which is about 1 cm. long. However, the activity is strongest at the exact place where the impulses are initiated and there is a gradient of local activity away from this region (fig. 2). This gradient agrees with that of automaticity.

To avoid misunderstanding it must be emphasized that not all slow potential changes in muscle are local potentials like those which have been described here. In cardiac muscle and in some smooth muscles there are in addition states of negativity which have been called residual negativity (14). These potentials differ from the local potentials by the fact that they always follow the process of conduction and that they are about as large as the spike potentials. Furthermore, slow potential changes may be recorded which are simply due to the fusion of spike potentials resulting from an unfavorable arrangement of the leads (14).

2. *The significance of the local potentials for the rhythmicity of smooth muscle.* In the ureter spontaneous contractions often follow each other at regular intervals of 15 seconds or longer. In this case each response is initiated by a slow rise in negativity. The conducted response produces repolarization, thereby restoring the previous condition of the pacemaker (fig. 4). Repolarization also follows the response to a stimulus. This observation explains why the pause following a premature response is as great as a normal pause, in agreement with the well-known result of similar experiments on the heart.

The contractions of the ureter may also occur in groups, separated by several minutes of complete rest. The frequency of the beats within one group is high, one beat every 3 to 4 seconds. This type of activity is always associated with the presence of oscillatory local potentials and it is due to the fact that several oscillations in succession set up conducted responses. At the end of each group there are further oscillations of gradually diminishing magnitude. The Luciani groups of cardiac muscle closely resemble the phenomenon just described. They are also due to the presence of oscillatory local potentials (15).

The grouping of responses in the ureter and the oscillatory local potentials probably are abnormal phenomena. Oscillations were found most frequently in preparations which had been kept in Ringer's solution for some hours before the observation. As a preparation deteriorates, the number of oscillations before the discharge increases (fig. 3B). There may also be oscillations of gradually increasing and decreasing magnitude which do not produce any conducted impulses.

3. *Inhibition in visceral muscles.* It is surprising that the local potential oscillations are largely independent from variations in muscular excitability.

This is best demonstrated by the action of adrenaline on the intestine. The drug greatly diminishes excitability and stops the conduction of impulses (12), but it does not significantly alter the local potentials. It has, furthermore, been shown that potential oscillations may continue in deteriorated preparations with undiminished intensity and frequency while no impulses are discharged, a condition indicating low excitability.

The observations just mentioned have an important bearing on the problem of inhibition in visceral smooth muscle. It has been concluded (12) from an entirely different series of observations that inhibition is entirely due to the experimentally demonstrated decrease in muscular excitability and to the consequent diminution or cessation of the discharge of impulses and not due to any decrease in the intrinsic stimulus for the discharge of impulses. This conclusion is confirmed by the fact that the local potentials are not abolished during inhibition. To explain the transitory excitatory action of adrenaline preceding the inhibition, as observed in the uterus of many species, it was assumed, furthermore, that adrenaline, besides decreasing excitability, causes an increase in the tendency for setting up impulses in uterine muscle. This assumption is confirmed by the observation of Balassa and Gurd (7) that adrenaline, although it inhibits motility, actually increases the size of the local potentials in the cat's uterus.

4. *Local potentials and tonus.* The results reported here are significant for the question of the tonus of smooth muscle. The absence of spike potentials indicates that the slowly developing weak contraction of the ureter previous to each spontaneous peristalsis is not a tetanic contraction. However, the difference between this continuous type of contraction and that due to conducted impulses probably lies only in the underlying excitatory mechanism, not in the mechanism of contraction.

Because the term tonus generally is used purely descriptively for any weak, sustained contraction, it should not be expected that the underlying mechanism is the same in all cases. It has been shown (13) that tonic contractions of visceral smooth muscles are often accompanied by a discharge of conducted impulses and are, therefore, not essentially different from tetanic contractions. It is not possible to distinguish this type of contraction from non-propagated activity except by a careful analysis of the accompanying electric changes.

It is probable that the tonus of visceral smooth muscles generally is predominantly due to a tetanic contraction because, in those cases which have been studied as yet, local activity, not accompanied by a discharge of impulses, is so weak that it does not produce any visible movements. It seems that the latter type of contraction has no significance for the motility of the muscle and is merely incidental to the membrane changes which are responsible for the initiation of muscular impulses. The close correlation between mechanical and electrical changes in the absence of conduction, as found in the ureter, presents an interesting, but as yet obscure, problem. This phenomenon may be taken as an indication that the ultimate causes of automaticity are fluctuations in metabolism.

## SUMMARY

The action potentials accompanying spontaneous contractions were studied in the ureter and the intestine. At the pacemaker each conducted impulse is preceded by a gradual rise in negativity. This electric change, which is much smaller than the spike potential, is non-conducted. In the ureter it is found only within a region of about 1 cm. from the origin of the impulses and it is strongest at the pacemaker itself. The local potentials can be interpreted as changes in the polarization of the cell surface. They can be considered as the basis of the automaticity of smooth muscle.

In the ureter the local potentials are either a gradually rising negativity or oscillatory changes of gradually increasing amplitude. In the latter case the impulses arise from the negative crest of some of the oscillations and they are often discharged in groups. The former type of local potential must be considered as the normal type because it is generally found in preparations in which the spontaneous motility is like that under normal conditions.

In the intestine slow potential waves are almost continuously present in all regions of the organ. Impulses are discharged, often in bursts, at the negative crest of such waves. The evidence indicates that the slow potentials are due to a non-conducted activity which has the function of initiating impulses.

In all the tissues studied local potential changes can take place while no impulses are discharged. This condition is produced by a depression of the excitability of the muscle, in the intestine, for instance, by adrenaline. The implications of this independence of local potentials from excitability for the question of the inhibition of smooth muscle are discussed.

The local potentials are not accompanied by visible movements of the muscle but in the ureter a small rise in tension, running closely parallel with the local potential, precedes each spontaneous contraction. Also, the contractions elicited by adrenaline are preceded by a slow rise in tonus.

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# THE ACTION POTENTIALS ACCOMPANYING CONDUCTED RESPONSES IN VISCERAL SMOOTH MUSCLES<sup>1</sup>

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It has been shown previously that a wave of contraction in visceral smooth muscles is accompanied by an action potential which usually consists of brief spikes similar to those of nerve and striated muscle, but also potential changes of longer duration like those of cardiac muscle were observed (5, 7). In every case the action potentials consisted of waves of negativity suggesting that the mechanism of conduction in smooth muscle is essentially the same as in other excitable tissues. The experiments reported here confirm these results. Furthermore, the slow phases of the action potentials, which may be large and of long duration, but could not be recorded satisfactorily by the apparatus previously used, were studied by means of a direct coupled amplifier.

The electric changes in smooth muscle as well as in any other tissue can be interpreted only under favorable experimental conditions. For this reason the precise meaning of many records of action potentials of smooth muscle reported in the literature remains uncertain. Most of these records show irregular oscillations superimposed on slow variations of the baseline. It must be emphasized that such slow potentials do not prove the existence of slow electric changes which are different and independent from spike potentials, even if the possibility of movement artifacts has been eliminated. Such potentials can be produced by the incomplete resolution of spikes which occurs if the effective width of the leads is greater than the distance between two impulses traveling along the muscle. The resulting fusion will become the more complete the greater the width and distance of the leads and the thicker the mass of tissue between the leads. This interpretation can be applied to the recent work of Bourdillon (3) and Bourdillon and Lidwell (4) on the action potentials of the rabbit's vagina. Whether the slow potential variations observed in this organ are due to a process different from spike potentials, as the authors suggest, cannot be decided without further analysis.

In comparing the action potentials of smooth muscle with those of nerve and skeletal muscle it is important to keep in mind the different dimensions in these types of tissues. In smooth muscle the width of the active region in a spike potential is less than 1 mm. Thus, during a peristaltic wave of the guinea pig's ureter the spikes follow each other at a distance of less than 1 mm. (7). It may be said, therefore, that 1 mm. in smooth muscle is equivalent to a distance of at least a few centimeters in nerve or skeletal muscle. This difference is explained

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by the small diameter of the fibers of smooth muscle. For the same reason electrotonic spread of potentials is slight as compared with nerve.

**METHODS.** Uterine strips from cats and guinea pigs during estrus and pregnancy, strips from the small intestine of the rabbit and cat, and the ureter of the cat, dog, rabbit, guinea pig and rat were used. In the cat, estrus was produced by injecting theelin (500 int. units) every two days for four (incomplete estrus) or five (complete estrus) days.

The muscles were prepared and mounted essentially as described previously (5). They were left in Ringer's solution for one-half to one hour before the start of the experiment. To prevent drying it was found advantageous, if very thin muscles were used, to wet the walls of the chamber with water or dilute Ringer's solution.

Very small calomel electrodes were used as leads. They made contact with the muscle by flexible thin cottonwicks (less than 1 mm. in width at the muscle), soaked in Ringer's solution. The potential changes were recorded by a direct coupled Thoenis amplifier and a G. E. oscillograph. The grid leads of the input were 100 megohm. Preliminary records were usually taken by a mechanical recorder on a smoked drum. If the preparations were carefully prepared and the surrounding tissue was completely removed the spike potentials generally had a magnitude of 4 to 6 mVolt.

The electric stimuli were condenser discharges ( $7_{\mu}\text{F}$ ) applied through platinum electrodes. Ringer-Locke's solution buffered by phosphate was used (0.9 per cent NaCl, 0.024 per cent  $\text{CaCl}_2$ , 0.042 per cent KCl). A solution of rather high Ca-ion concentration is essential for maintaining excitability and for obtaining strong contractions.

**RESULTS.** *Interpretation of monophasic and diphasic potentials.* Monophasic action potentials were obtained if the distant lead was on an inactive region of the muscle. This was usually accomplished by pinching the preparation between the leads. The potentials were partly diphasic unless they were led off from a point less than 1 mm. from the injured region. That this distance has to be much shorter than in nerve is to be expected on the basis of the electric conditions in the tissue (cf. 1, 10) if it is considered that the length of the active region during the conduction of an impulse is usually less than 1 mm.

As a general practice the proximal lead was placed directly on the margin of the injured region. If the lead was shifted so that it partly covered this region, the potentials did not change their character but were weaker. If the lead was placed on intact tissue more than 1 mm. away from the injury the potentials became somewhat greater, but had a rapid downstroke and subsequent hump in the falling phase, or a brief positive wave following the spike. These variations were considered as diphasic artifacts and resemble corresponding phenomena in nerve. The potentials remained strictly monophasic for only a short time. After a few minutes they gradually became more and more diphasic. For this reason, all the monophasic records described here were obtained within 2 minutes after injury.

The monophasic potentials show a great diversity. They may consist of

single brief spikes (fig. 1C), a repetitive discharge of such spikes (fig. 3C), or a long-lasting negativity (fig. 2D). In view of the necessity of placing the leads

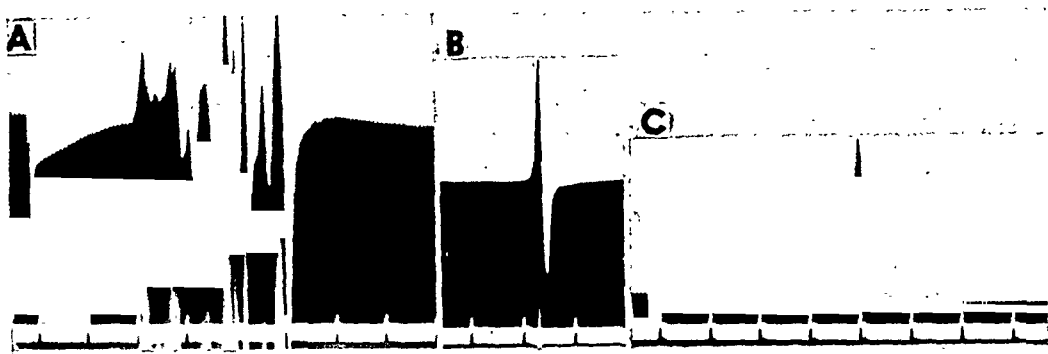


Fig. 1. Potentials from a uterine strip of the cat, an example of a brief impulse. The cat was in incomplete estrus. A: diphasic with long distance (2.2 cm.) between leads. Distance between stimulating cathode and first lead 1.5 cm. The last downstroke is due to the passage of the impulse across the second lead and should be compared with the monophasic potential. First downstroke stimulus artifact. B: Diphasic with short distance (1 mm.) between leads. Note absence of T-wave. C: monophasic. Temp. 34°. Time  $\frac{1}{5}$  sec.

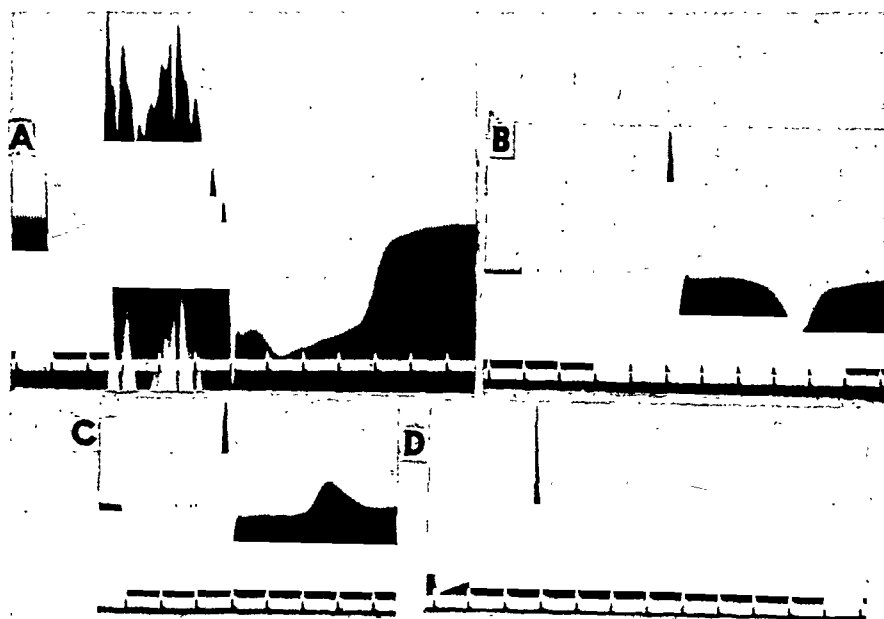


Fig. 2. Potentials from the ureter of the rat showing a long continued state of negativity. A: Diphasic potential with long distance between leads (2.1 cm.). The rapid downstroke in the middle of the record indicates the arrival of the impulse at the second lead. B: Diphasic with short distance (1 mm.) between leads, showing R- and T-waves. C: The same but from other region. Positive T-wave. D: Monophasic. Temp. 34°. Time  $\frac{1}{5}$  sec.

very close to the injury, the possibility that these monophasic action potentials are abnormal must be examined. Diphasic records are useful for this purpose.

Because of the shortness of the active region it is usually possible to separate the electric changes occurring at the two leads. With widely separated leads, it may be expected that the positive wave of the diphasic potential is a relatively undistorted record of the potential change at the distal lead and that it should resemble the monophasic potential, because an impulse cannot have any noticeable effect after it has passed beyond the leads. In agreement with theoretical expectation this last phase of the diphasic potential is roughly the mirror image of the monophasic potential for a given preparation, as shown in figures 1, 2 and 3.

The first part of the diphasic potential obtained with widely separated leads consists of a rapid upstroke followed by highly irregular, but exactly reproducible, potential variations. That these irregularities are due to local conditions along the path of conduction is shown by the previous observation that the various peaks of the potential record can be eliminated one by one by blocking con-

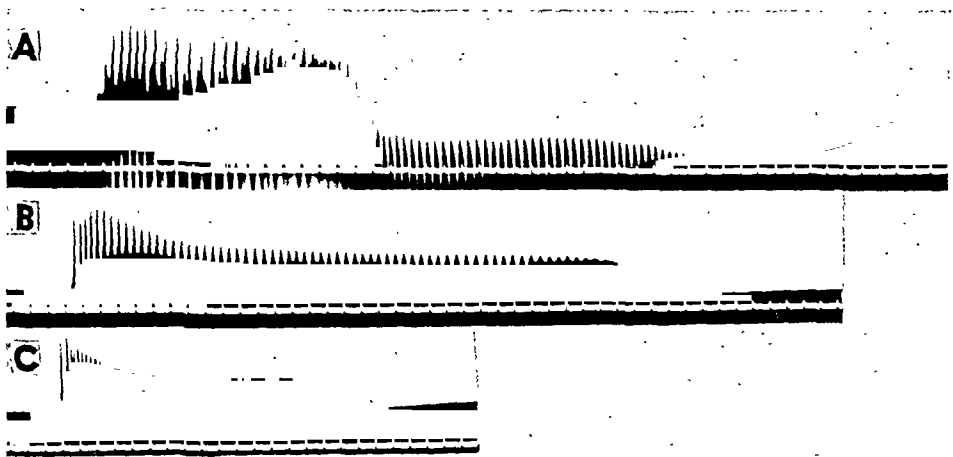


Fig. 3. Potentials from ureter of guinea pig showing a repetitive response of high frequency and residual negativity at the end of the discharge. A: Diphasic with long distance between leads (2.8 cm.). B: Monophasic from same preparation as A. C: Monophasic from another preparation. Temp. 30°. Time  $\frac{1}{5}$  sec.

duction at various places between the leads, progressing from the distal to the proximal lead (5). This result was confirmed by placing the distal lead gradually closer to the proximal lead. Under these conditions the positive phase of the diphasic potential remained unchanged but the first, irregular, phase became progressively shorter.

To explain the diphasic potential, it has been suggested (9) that the smooth muscle preparations behave like a series of muscular units, a condition which could arise from the varying width of the anastomoses between the muscle fibers. However, one would expect, then, that the separation into units also manifests itself in other respects. For instance an electric current should produce polarization not only close to the electrodes but also at regions where the anastomoses are very narrow. On the contrary it was found that electric current stimulated at only one electrode, even if the stimulus was many times above threshold.

It is more probable that the irregular variations accompanying the conduction

of the impulse between the leads is caused by local asymmetries of the preparation, due to attached extraneous tissue, local injury, etc. That such conditions can produce phenomena like those described here has been shown for nerve (cf. 1, 10) but the effects are even more conspicuous in smooth muscle because the distance between the leads was very large as compared with the length of the active region.

*Comparison of the action potentials of different muscles.* The rising phase is brief in all preparations. The peak is reached in 0.05 to 0.1 second. These values, however, do not indicate the true time relations of the potential change, because most or all of this time interval is accounted for by the conduction over the effective width of the lead. The fact that the frequency of discharge is as high as 40 impulses per second in one of the preparations used indicates that the duration of the spike process alone is less than 25 msec. and it may possibly have the same order of magnitude as in nerve.

The total duration of the action potential, on the other hand, may be long and it varies within wide limits in different muscles. It was shortest in the uterus of the cat during incomplete estrus (fig. 1), and in the small intestine. The potential has a brief spike, but the return to the baseline is slow. It is similar in its time relations to that of skeletal muscle (cf. Bishop, Young).

The other extreme is the ureter of the rat (fig. 2). After an initial spike a considerable negativity is maintained at a fairly constant level for various periods of time up to 10 seconds. The duration seems to depend largely on excitability. Cocain, which raises electric excitability, particularly in depressed preparations, prolongs the action potential. The similarity with the action potential of cardiac muscle is striking. In diphasic potentials, a T-wave, often positive, is present. If the distance between the leads is varied the duration of the R-wave varies proportionately, but the R-T interval remains unchanged. The same type of action potential, but somewhat shorter in duration, was observed in the ureter of all the other species studied (cat, dog, rabbit), with the exception of the guinea pig.

The initial spike of the monophasic potential of the ureter probably is not a diphasic artifact because it is always present in the positive wave of the diphasic potential (fig. 2). In monophasic potentials the spike sometimes was absent and the upstroke slower than normally only if the lead was several millimeters from the block. Pinching the preparation directly below the lead, then, restored the normal character of the potential.

The ureter of the guinea pig (fig. 3) presents intermediate stages between the types of action potential just described. A single stimulus produces a rhythmic discharge of brief spikes, but a state of negativity may persist after the termination of the spike discharge. The frequency may be as high as 40 impulses per second (38°) in highly excitable preparations and it gradually declines during each response. The frequency of discharge is also lower during the refractory phase and under the influence of low calcium Ringer's solution.

The spikes are superimposed on a rising baseline. This phenomenon is partly explained by the fact that the impulses follow each other at a distance of only 1 mm. or less. If the effective width of the lead is greater than this distance



a partial fusion results. However, the fact that a state of negativity often persists at the end of the discharge without any oscillations shows that the rise in the baseline is not entirely due to fusion. An extreme example of this condition is shown in figure 13C, where the rhythmic phase of the action potential appears insignificant. This potential differs only slightly from that of the rat's ureter.

The monophasic action potentials are quite characteristic for the different muscle preparations used. However, in some cases considerable variations were observed. In two out of more than fifty preparations of the rat's ureter, the discharge was repetitive, similar to that of the guinea pig but with a lower frequency of discharge (fig. 4). Furthermore, if the plateau was maintained for several seconds, often the negativity suddenly disappeared and a series of distinct



Fig. 4. Monophasic potentials from a preparation of the rat's ureter showing an unusual diversity of electric responses. They were obtained from different regions within 2 hours. Temp. 34°. Time  $\frac{1}{5}$  sec.

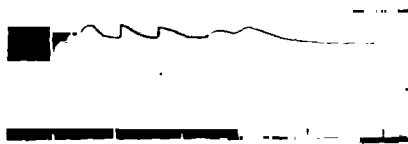


Fig. 5. Monophasic action potentials from uterine strip of pregnant cat. Discharge elicited by electric shock. The records indicate considerable residual negativity after each wave of activity. Mechanical recorder. Temp. 36°. Time in seconds.

arge spikes were following. Other peculiar rhythmic variations in the action potential of the rat's ureter occasionally observed are shown in figure 4.

Uterine and intestinal strips, although they normally give a repetitive discharge during spontaneous activity (6), usually give only a single brief impulse when a monophasic lead is used, evidently as the result of the injury close to the lead. In strips from the pregnant uterus of the guinea pig, however, a very irregular monophasic potential, beginning with a spike and followed by a high but rather irregular plateau, sometimes was obtained (fig. 5). It is improbable that such potentials occur under normal conditions because diphasic potentials of spontaneous contractions show a fairly regular discharge with a frequency as high as five impulses a second in the uterus and ten impulses per second in the intestine.

A weak positive after potential lasting for 10 to 20 seconds was often, but not invariably, present in the various types of muscle studied.

**DISCUSSION.** The action potentials of visceral smooth muscles show a much greater diversity than those of other types of muscles. To obtain a unified concept of these potentials two components may be distinguished: a spike potential, which invariably is present in conducted responses; and residual negativity which follows the initial spikes and lasts much longer than the former. The differences between the muscles, then, are considered as due to the prominence of one or the other of these two components. In the action potentials of uterine and intestinal muscle the spike is the most conspicuous feature. The time relations of these potentials resemble closely those of skeletal muscle. In the ureter, however, the spike usually is followed by a high plateau which may be maintained for many seconds. This type of potential resembles closely that of cardiac muscle. There are also intermediate types of potentials showing spikes superimposed on a considerable residual negativity.

The differences in the character of the potentials can be correlated with other physiological differences between the muscles. Uterine and intestinal muscle, which has a weak residual negativity, has a relatively short refractory phase and repetitive stimulation produces a tetanus (5). The ureter, on the other hand, corresponding to the long duration of its potential, has a long refractory phase and does not show summation (5), also in this respect resembling cardiac muscle.

The comparison between different action potentials suggests that the residual negativity, if it is of sufficient magnitude suppresses and takes the place of the spike potentials. It appears probable, therefore, that the state of negativity as observed in the ureter and in cardiac muscle is an excitatory state not essentially different from the spike process.

Records of the isometric contraction of short pieces of the ureter clearly show that the rising phase of the mechanical response lasts much longer than the spike of the action potential. It is probable, therefore, that the slow phase of the action potentials is an accompaniment of the production of mechanical energy as has been suggested by Bishop and Gilson for skeletal muscle. It would not be justified, however, to conclude that the process of contraction itself is giving rise to electric phenomena. At the present state of our knowledge it can only be stated that the degree of polarization of the cell surface probably is closely related to the metabolic changes within the cells. This conclusion is confirmed by the tonus changes which accompany non-conducted potential changes in smooth and cardiac muscle (8).

#### SUMMARY

Monophasic action potentials accompanying the conducted responses of uterine and intestinal muscle and of the ureter were recorded. The results obtained were confirmed by an analysis of diphasic potentials recorded with widely separated leads.

At the time of arrival of an impulse at the first lead there is a rapid rise in negativity. In other respects the potentials show a great diversity in different

muscles. The monophasic potential is either a brief spike, a long continued negativity or a repetitive discharge. One can group the potentials into a continuous series by distinguishing two components, the spike and residual negativity. The latter component is small in some cases, giving action potentials similar to those of nerve and skeletal muscle. In the ureter of most species, on the other hand, the residual negativity following a single spike is maintained at a considerable magnitude for periods as long as 10 seconds, giving an action potential like that of cardiac muscle. The ureter of the guinea pig is an intermediate type where both components are equally important.

Several facts suggest that residual negativity is a state of activity which is not essentially different from the spike and can take the place of the latter.

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# THE ACTIVITY OF THE DESCENDING DUODENUM DURING NAUSEA<sup>1</sup>

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Nausea is often accompanied by manifestations of vegetative nervous system activity such as pallor and sweating. That the smooth muscle of the intestines likewise undergoes changes during nausea is a reasonable assumption, especially since nausea and vomiting are such closely related phenomena. To test the validity of this assumption with respect to the duodenum, records of duodenal activity were taken during artificially produced nausea.

**PROCEDURE.** Subjects for the experiments were 5 normal volunteers and 15 patients. Of the patients, 10 had no organic gastro-intestinal or neurological disease, while in 5 the respective diagnoses were: healed gastric ulcer, minimal hepatitis, syringomyelia, labyrinthitis and myxedema.

The subjects were intubated with the double lumen tube described by Miller and Abbott (1). Each lumen led to one of two tandem balloons which individually held 45 to 50 cc. when filled but not distended with air. In this state, each balloon measured 4.5 to 5.0 cm. in length and 3.5 cm. in diameter. Kymographic records of the intestinal activity were taken with the apparatus used by Ingelfinger and Abbott (2). This apparatus primarily measures volumetric changes in the balloons, since the air in the balloons is kept under more or less constant pressure (10–20 cm. of water).

The two tandem balloons were passed into the descending duodenum under fluoroscopic observation. They were kept in place by a technique which takes advantage of the fact that a single balloon, if inflated proximal to the mid-point of the descending duodenum, is expelled backwards into the stomach; if inflated distal to the mid-point, it is pushed ahead into the transverse duodenum and jejunum (3). Two balloons may therefore be “anchored,” provided that they are inflated simultaneously when one balloon lies proximal, the other distal to the midpoint of the descending duodenum (fig. 1). In two cases this technique was not successful, and the tandem balloons had to be “anchored” by inflating the proximal balloon in the gastric antrum.

After a control record of the duodenal activity was taken, the labyrinth was stimulated by gentle irrigation of the external auditory canals with 30 cc. of either cold (18°) or warm (45°) water. Although this method failed to produce nausea in 9 of the 20 subjects, it possesses certain advantages: 1. Nausea, when produced, is very likely of “central” origin. 2. The side reactions of drugs are avoided. 3. The nausea elicited is usually not severe enough to

<sup>1</sup> Presented in part before the Philadelphia Physiological Society—February, 1942.

provoke the violent muscular contractions of emesis, which interfere with the recording of the intrinsic intestinal activity.

To 5 of the subjects in whom repeated caloric stimulation of the semicircular canals failed to produce nausea, 0.010 gram morphine sulfate was given subcutaneously.

**RESULTS.** Caloric stimulation of the labyrinth elicited nystagmus and vertigo in all subjects, while varying degrees of nausea were produced 13 times in 11

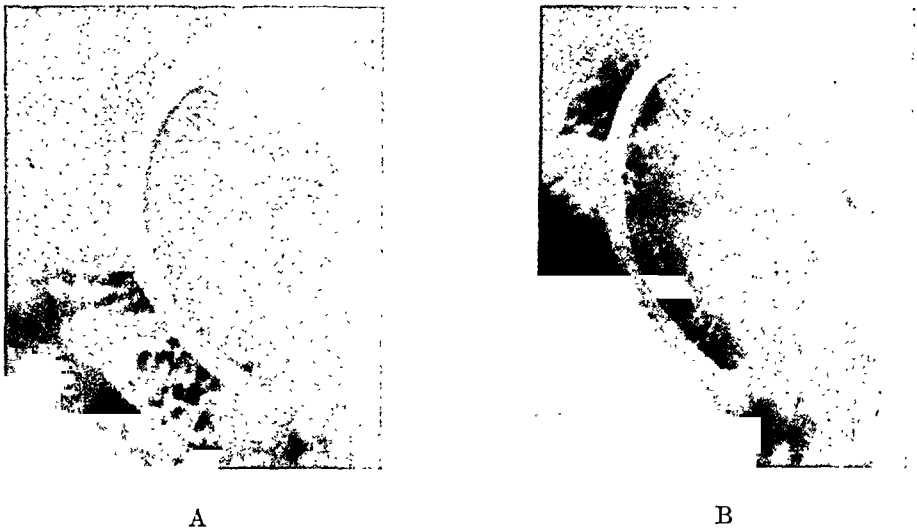


Fig. 1. Roentgenographic demonstration of the tandem balloons in the descending duodenum. A. The duodenum during a quiet phase. B. Contraction waves compressing the distal part of the proximal balloon and the middle of the distal balloon. These waves are progressing down the intestine.

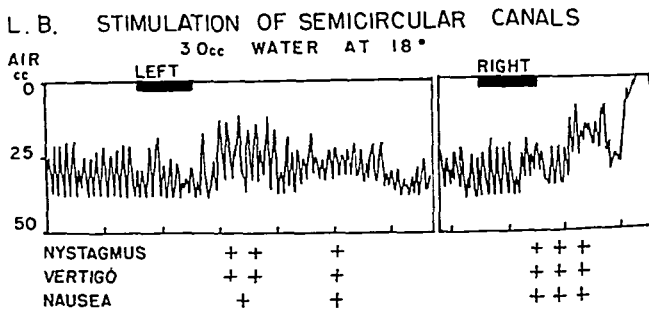


Fig. 2. Record of distal balloon. Time units are minutes. Each of the waves occurring 8 to 9 times per minute is produced by one of the ring-like contractions shown in figure 1, B. After stimulation of the right labyrinth, both the nausea and the duodenal contraction are more marked.

individuals. In 8 of these instances, the descending duodenum underwent a generalized contraction simultaneously with the onset of nausea, the degree and the duration of the contraction being roughly proportional to the intensity and duration of the nausea produced (fig. 2). The usual interval between the beginning of caloric stimulation and the onset of nausea and duodenal spasm

was 1 to  $2\frac{1}{2}$  minutes, but in one case these reactions occurred almost immediately. The duodenal contraction, which failed to displace the balloon in 3 instances, persisted for  $3\frac{1}{2}$ , 6, and 5 minutes respectively. A strong reaction to labyrinthine stimulation was characterized by a complete obliteration of the duodenal lumen (fig. 3), whereas the mildest response observed in these subjects displaced 5 cc. of air from the recording balloon.

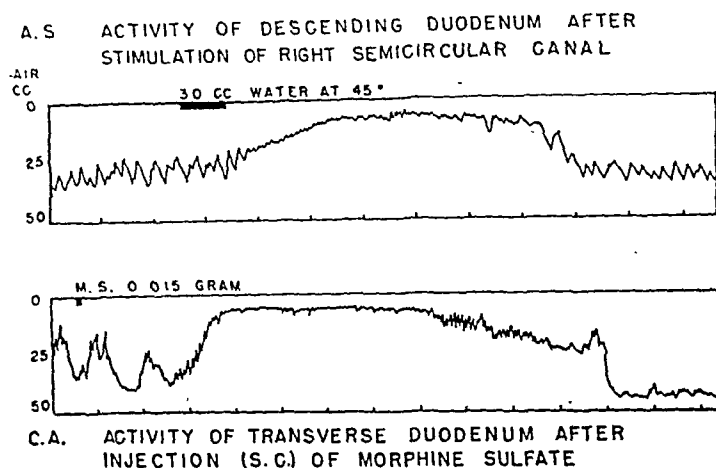


Fig. 3. Records from 2 subjects showing the similarity of a strong duodenal response to caloric excitation of the labyrinth and to the administration of morphine sulfate. Following the contraction induced by morphine, the duodenum is relaxed.

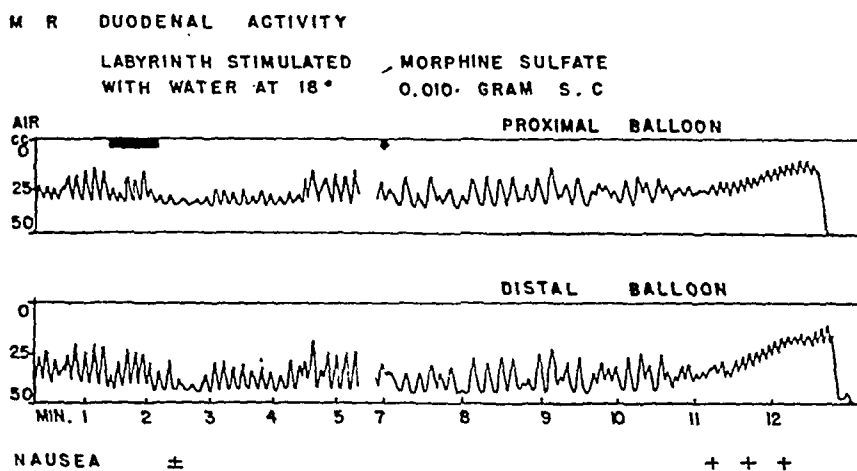


Fig. 4. Simultaneous records of the proximal and the distal balloon. Relaxation of the duodenum during mild nausea following caloric stimulation. Simultaneous contraction of the proximal and distal portions of the descending duodenum following administration of morphine sulfate. Balloons filled with air as they were expelled into stomach.

In 3 subjects, who experienced rather equivocal symptoms of nausea, duodenal activity was decreased in that the duodenum relaxed slightly and the peristaltic waves showed diminished amplitude (fig. 4).

In 2 subjects who vomited after caloric stimulation, the record of duodenal activity was obscured.

Nine subjects, including 3 normal volunteers and 6 patients, failed to evidence nausea after stimulation of the semicircular canals. In these, the record of duodenal activity remained unchanged, even though vertigo was often pronounced.

All the individuals who were given morphine sulfate subsequent to labyrinthine stimulation experienced severe nausea, during which the duodenum contracted strongly. These reactions occurred from  $1\frac{1}{2}$  to  $3\frac{1}{2}$  minutes after administration of the drug. The records of duodenal contraction during the nausea following the administration of morphine and that following labyrinthine stimulation were quite similar (fig. 3).

As judged from fluoroscopic observation and, more accurately, from the simultaneous recording of both balloons (fig. 4), the contraction which involved the descending duodenum during nausea occurred at the same instant in the proximal and the distal portions. Reverse peristalsis involving first the distal, then the proximal balloon was never observed. Nevertheless, the duodenal contraction expelled both balloons into the stomach in 9 instances without any vomiting taking place. This occurred 6 times after labyrinthine stimulation and 3 times after morphine sulfate was given. As the balloons passed backwards through the pylorus, each contained air in amounts varying between 5 and 15 cc.

**DISCUSSION.** The rôle of the duodenum during nausea and vomiting has generally been neglected. Hatcher (4) reviewed the mechanism of vomiting in 1924 but failed to mention the duodenum. He apparently felt that pylorospasm initiated the gastro-intestinal reactions during emesis. Best and Taylor (5) emphasize antiperistalsis and "tension upon" the walls of the esophagus, stomach and duodenum. On theoretical grounds, Alvarez (6) has suggested that at times nausea "has its origin in reverse peristalsis in the bowel" but apparently was never able to confirm this hypothesis. Recently Oppenheimer and Mann (7), working on dogs with exteriorized intestinal loops, showed that attacks of emesis provoked by drugs were preceded by a short increase in intestinal activity.

The problem of seasickness has led a few otolaryngologists to investigate the relation between labyrinthine stimulation and intestinal activity. According to Spiegel and Demetriades (8), who studied the surgically exposed intestinal loops of animals, *caloric stimulation of the labyrinth* increased the extent of small intestinal contractions. LeHeux and deKleyn (9) found that labyrinthine extirpation in the cat enfeebled gastric and, to a lesser degree, intestinal motor activity. These observations, however, have not been applied to the field of gastro-intestinal physiology.

The present experiments show that nausea, whether caused by excitation of the semicircular canals or by the administration of morphine, is frequently accompanied by a generalized contraction of the descending duodenum. The question arises whether some of the symptoms of nausea, such as the desire to vomit, may actually be caused by the duodenal contraction. Though possible, this hypothesis is unlikely since we have shown that a narrowing of the duodenal

lumen does not always attend nausea. Furthermore, as Abbott and Pendergrass (10) have reported, the administration of 0.015 gram of morphine causes small intestinal contraction, but clinically this same dose does not invariably nauseate the patient. Our subjects experienced nausea without fail after receiving morphine, but it must be remembered that they were intubated and had been exposed to labyrinthine stimulation.

Recent experiments by Quigley et al. (11) and by Mecray (12) have corroborated Abbott's (13) contention that reverse peristalsis—i.e., oral progress of a muscular contraction—is rarely seen, particularly in the human small intestine. Our records indicate that during nausea balloons in the descending duodenum are pushed backwards into the stomach even though the "peristaltic" waves continue to travel aborally. A possible explanation of this phenomenon is that the intestinal gradient postulated by Alvarez (14) has been reversed by the generalized but stationary duodenal contraction.

The state of the pylorus is hard to determine by our methods, but obviously complete pylorospasm is not present during nausea or just before emesis. If it were, the duodenum could not expel the partially inflated balloons as easily as it does.

Since the nausea and duodenal contraction produced by labyrinthine stimulation must be of "central" origin, a similar duodenal response may characterize the gastro-intestinal reactions of intra-cranial disorders, of sea or air-sickness, and of migraine. "Bilious spells"—attacks consisting of headache, nausea, and the vomiting of bile-stained material—may also depend upon a "central" mechanism which brings about duodenal spasm and regurgitation of intestinal contents. These "bilious spells," especially when they are accompanied by right upper abdominal distress, are sometimes treated with opiates, but the experiments here presented show why such therapy merely prolongs the symptoms.

#### SUMMARY

Kymographic records of duodenal activity after labyrinthine excitation and after the administration of morphine sulfate were taken in 20 subjects.

Nausea was produced 13 times by caloric stimulation of the labyrinth and 5 times by morphine sulfate administration. In 13 of these instances, a generalized contraction of the descending duodenum was recorded during nausea.

The contraction of the descending duodenum often expelled both balloons backward into the stomach, although no reverse peristalsis was observed.

It is suggested that duodenal spasm is a frequent concomitant of nausea, and that this spasm pushes the duodenal contents into the stomach by reversing the intestinal gradient. Necessarily, absolute pylorospasm during nausea would then be impossible.

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# DIFFERENCES AMONG ADRENAL STEROIDS WITH RESPECT TO THEIR EFFICACY IN PROTECTING THE ADRENALECTOMIZED DOG AGAINST CIRCULATORY FAILURE

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In previous communications (1, 2) it was pointed out that the synthetic steroid desoxycorticosterone acetate differs qualitatively from adrenal cortical extract with respect to its efficiency in protecting the circulation of the adrenalectomized dog against various shock inducing procedures. For example, stripping the intestine or removal of both adrenal glands at a single stage operation, leads to a slow but steady decline in arterial pressure of both the untreated and desoxycorticosterone treated dog, death occurring within 8 to 24 hours. If, however, the animals are treated with liberal amounts of potent extract, the blood pressure fall is negligible, and normal activity and vigor are maintained. It is evident therefore, that whole extract, unlike desoxycorticosterone, contains factors which afford protection to the circulation of the adrenalectomized dog subjected to these two procedures.

Failure of the circulation which is unresponsive to treatment with desoxycorticosterone is apparently unrelated to alterations in the electrolyte pattern of the blood, renal loss of body fluids, and hemoconcentration. Lowering of the blood sugar is a fairly constant finding following the single stage bilateral adrenalectomy, less so after stripping the intestine. In some cases, however, hypoglycemia may develop. The experiments show that no simple correlation exists between the actual level of blood sugar and the decline in arterial pressure. Intramuscular injections of glucose at frequent intervals and in amounts sufficient to maintain the blood sugar at or near normal, will prolong survival although the blood pressure is not restored. It is probable therefore that marked alterations in carbohydrate metabolism are characteristic responses to these two procedures, the fall in blood sugar merely serving as a rough index of upsets of a more fundamental nature in the metabolism of glucose.

Since desoxycorticosterone is practically without effect upon those phases of carbohydrate metabolism influenced by cortical extract and adrenal steroids having an oxygen at carbon-11, and since it is also ineffective as a prophylactic against types of shock in which blood sugar changes are marked, it was considered essential to repeat earlier experiments (1, 2) using corticosterone and 17-hydroxy-11-dehydrocorticosterone.<sup>2</sup>

<sup>1</sup> Upjohn Research Fellow

<sup>2</sup> We are greatly indebted to Dr. E. C. Kendall, Division of Biochemistry, Mayo Foundation for generous supplies of Compound E.

The corticosterone was obtained from Dr. J. J. Piffner, Parke Davis and Co. and Dr. Oscar Wintersteiner, Squibb Institute for Medical Research. Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products, Inc. furnished the desoxycorticosterone acetate (Percorten).

The type of animal used and the methods employed for inducing circulatory collapse, and determining blood pressure, have been discussed in earlier papers (3, 4). The corticosterone and 17-hydroxy-11-dehydrocorticosterone (compound E of Kendall's series) were dissolved in 95 per cent alcohol and then diluted with distilled water so that each 1.75 cc. contained 5 mgm. of steroid. This 50 per cent alcohol solution was injected intravenously at a very slow rate. Tests made on both intact and adrenalectomized dogs afforded no evidence that the alcohol *per se* affected the results.

I. *Trauma to the intestine.* The entire length of the small intestine was gently stripped between the fingers for twenty-five minutes using strict asepsis. In the normal dog, this amount of stripping is without demonstrable effect upon the blood pressure or vigor of the animal. The adrenalectomized dog maintained in apparently normal health by cortical extract is extremely sensitive to such treatment, and dies from circulatory collapse within 8 to 19 hours. Fore treatment with desoxycorticosterone is without effect upon the circulation in this type of trauma.

A. *Effect of 17-hydroxy-11-dehydrocorticosterone when used as a prophylactic fore treatment.* So far as the writers are aware no one has tested this steroid in shock produced by trauma to the intestine. Representative data obtained from study of four dogs are given in table 1. The animals at no time exhibited symptoms, remaining active and vigorous from the time of recovery from the anesthetic until the experiment was discontinued. Hemoconcentration occurred in all the animals despite lack of symptoms. The blood sugar levels were elevated above normal. Animals treated with similar doses of desoxycorticosterone usually show a sharp decline in blood sugar and may exhibit hypoglycemic convulsions.

B. *Effect of corticosterone when used as a prophylactic fore treatment.* Selye and co-workers (5) reported that corticosterone will prolong the survival of intact rats shocked by crushing the intestine several times with hemostats. In view of this report, positive responses were anticipated with this steroid so that we were disappointed to find the results quite variable. Of four dogs subjected to intestinal stripping, two responded to the treatment very well, exhibiting no signs of circulatory failure. The blood pressure declined somewhat, but later recovered spontaneously to levels approaching normal (dog 4, table 1). The other two dogs developed circulatory failure and died within 12 hours. These animals reacted as though they had received no treatment whatever. The fact that 50 per cent of the dogs responded to corticosterone whereas 50 per cent did not might be an indication that the method of administration was at fault, i.e., that the corticosterone, unlike 17-hydroxy-11-dehydrocorticosterone, sometimes failed to remain in solution when injected directly into the blood, and was therefore not immediately available to the animal. Whatever the cause for the inconstant results, it must be concluded at present that the action of corticosterone is definitely inferior to that of 17-hydroxy-11-dehydrocorticosterone.

II. *Bilateral adrenalectomy at a single stage operation.* The dog reacts unfavorably to bilateral removal of both adrenal glands at a single stage operation unless

special care is taken to avoid injury to the nervous elements adjacent to the glands (2). The animals develop circulatory failure, rarely surviving for more than 10 to 20 hours. Treatment with desoxycorticosterone does not prolong the life span. If, however, the adrenals and surrounding tissue are thoroughly infiltrated with procaine previous to gland removal, so that afferent impulses arising in the area of injury are locally blocked, circulatory changes do not occur

TABLE 1

*Effect of adrenal steroids\* in protecting against circulatory failure following a twenty-five minute intestinal stripping*

DATE	TIME	BLOOD PRESSURE	PULSE	HEMO-GLOBIN	BLOOD SUGAR	REMARKS
Dog 1, 10.5 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone						
9/29	12:45 p.m.	mm. Hg 106	per minute 50	grams per cent 13.1	mgm. per cent 83	Completed stripping
	5:40 p.m.	105	152	16.7	60	
9/30	11:40 p.m.	102	124	14.6	89	Active
	11:40 a.m.	112	110	15.0	99	Normal, given food and water
Dog 2, 11.1 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone						
10/28	10:30 a.m.	105	100	9.0	85	Completed stripping
	3:30 p.m.	105	168	11.5	98	
10/29	9:30 p.m.	104	180	11.1	107	No symptoms
	10:30 a.m.	104	160	11.5	92	Normal, given food and water
Dog 3, 9.1 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone						
11/21	10:30 a.m.	112	120	14.3	83	Completed stripping. Pressure fell to 62 mm. Hg
	3:30 p.m.	86	196	15.6	60	
11/22	10:30 p.m.	96	168	15.0	96	Strong, given food
	10:30 a.m.	102	148	15.0	88	Appears normal
Dog 4, 11.2 kgm., 20 mgm. corticosterone						
1/10	11:30 a.m.	109	68	12.5	70	Completed stripping
	4:30 p.m.	112	104		71	
1/11	11:30 p.m.	98	188	19.8	66	Strong, active
	9:30 a.m.	93	192	15.3	70	Appears normal

\* Given in four intravenous injections of 5 mgm. each at 2 hours before the stripping, and at 2, 5 and 8 hours after the stripping.

and the animals can be easily maintained on desoxycorticosterone. The same thing is true if the spinal cord is sectioned at the level of the first thoracic vertebra previous to adrenal removal.<sup>3</sup>

A. *Efficacy of 17-hydroxy-11-dehydrocorticosterone when used as a prophylactic fore treatment.* Four dogs, given 10 to 20 mgm. in divided doses (table 2), were

<sup>3</sup> Unpublished work.

subjected to bilateral adrenalectomy at a single stage operation. All remained active and vigorous throughout the experimental period of 24 hours, an interval deemed adequate since untreated dogs subjected to this operation always succumb before the 24th hour. The only animal which showed a blood pressure decline was the one that received the least amount of steroid (table 2, dog 7).

TABLE 2

*Effect of adrenal steroids\* in protecting against circulatory failure following single stage bilateral adrenalectomy*

DATE	TIME	BLOOD PRESSURE	PULSE	HEMO- GLOBIN	BLOOD SUGAR	REMARKS
Dog 5, 11.1 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone						
10/2	11:00 a.m.	120	100	13.0	89	Completed operation
	4:00 p.m.	128	104	12.4	77	
10/3	11:00 p.m.	114	96	12.7	99	No symptoms
	11:00 a.m.	120	103	13.0	80	Normal
Dog 6, 8.6 kgm., 15 mgm. 17-hydroxy-11-dehydrocorticosterone						
10/6	12:10 p.m.	98	96	11.1	84	Completed operation
	5:00 p.m.	115	126	11.7	79	
10/7	11:00 p.m.	111	108	11.2	100	No symptoms
	11:00 a.m.	109	128	11.1	86	Normal
Dog 7, 10.7 kgm., 10 mgm. 17-hydroxy-11-dehydrocorticosterone						
10/27	4:00 p.m.	122	112	18.5	88	Completed operation
	10:00 p.m.	80	175	17.0	87	Lethargic
10/28	10:00 a.m.	86	180	16.8	85	Inactive. Given cortical extract
10/29	10:00 a.m.	105	120	17.0	86	Appears normal
Dog 8, 9.7 kgm., 20 mgm. corticosterone						
10/29	11:00 a.m.	105	120	13.8	87	Completed operation
	4:00 p.m.	83	184	14.0	90	
10/30	10:00 p.m.	80	240	13.9	96	Inactive
	10:00 a.m.	98	160	13.7	94	Appears normal

\* Given intravenously as follows: All dogs received 5 mgm. 2 hours before operation; dogs 5 and 8 received 3 injections of 5 mgm. each at 2, 5 and 8 hours after operation; dog 6 received 5 mgm. at 2 hours and 2.5 mgm. at 5 and 8 hours after operation; dog 7 received 2.5 mgm. at 2 and 5 hours after operation.

Despite the lowered pressure the dog remained active and alert. The response of this animal makes it seem evident that 10 mgm. represents approximately the minimum dose requisite for adequate protection against circulatory failure.

Blood studies revealed no indication of hemoconcentration. The blood sugars were elevated above pre-operative levels.

B. *Efficacy of corticosterone as a prophylactic fore treatment.* Sufficient cor-

ticosterone was available to test only one dog (table 2, dog 8) but this case proved strikingly clear. The arterial pressure fell 25 mm. Hg during the critical part of the experiment (8-12 hrs. following operation) but recovered without further treatment. The animal remained in good condition and ate full rations at the end of 24 hours.

There was no appreciable hemoconcentration as indicated by hemoglobin determinations. The blood sugar was elevated. The protection afforded this single corticosterone-treated dog seemed distinctly inferior to that given by equivalent amounts of 17-hydroxy-11-dehydrocorticosterone.

III. *Trauma to muscle masses.* It was next considered of interest to test the efficiency of 17-hydroxy-11-dehydrocorticosterone in protecting the circulation in those types of shock previously shown to respond well to desoxycorticosterone (1).

Trauma to muscle masses by 100 light blows with a wooden mallet to the hind leg of the deeply anesthetized adrenalectomized dog receiving minimum maintenance doses of cortical extract, results in circulatory failure within 8 to 14 hours. Fore treatment of similar animals with 20 mgm. of desoxycorticosterone prevents any impairment of the circulation.

Owing to the limited supply of 17-hydroxy-11-dehydrocorticosterone available, only two animals were tested (table 3). However, the results were so decisive that more are probably unnecessary to establish the point. Upon recovery from the ether the animals were strong, active and showed no significant changes in arterial pressure. The injured leg rapidly swelled to nearly twice normal size and some hemoconcentration was present. The blood sugar was elevated above normal.

IV. *Ineffectiveness of desoxycorticosterone acetate as a prophylactic for muscle injury when the adrenalectomized dog has been maintained on synthetic steroid instead of natural extract.* It was mentioned earlier that daily maintenance doses of cortical extract (0.3-0.5 cc. per kgm. body weight in which 1 cc. contains the equivalent of 50 grams of fresh cortex) will not prevent the circulatory failure which follows muscle trauma. If the animal is given 10 to 20 mgm. of desoxycorticosterone as fore treatment, 12 to 18 hours before trauma, and extract discontinued, it is able to withstand very severe tissue abuse without symptoms appearing (1). Recently we discovered, quite by accident, that the protective action of desoxycorticosterone is manifest *only in those animals which have been maintained on natural extract for several days previous to injury.* Desoxycorticosterone loses its protective effect upon the circulation when employed as a prophylactic on animals which have been maintained solely on this steroid and hence are completely lacking in the factors concerned with carbohydrate metabolism. Table 3 is a summary of the experimental findings. It will be noted that the blood sugar fell to low levels along with the blood pressure. Definite hypoglycemic symptoms were not observed.

V. *Efficacy of 17-hydroxy-11-dehydrocorticosterone as prophylactic fore treatment for circulatory failure following hemorrhage.* The adrenalectomized dog, receiving maintenance doses of extract and given fore treatment with desoxy-

corticosterone, responds to hemorrhage in all observable respects like the intact dog (2). Large quantities of blood (30-35 cc. per kgm.) can be withdrawn with-

TABLE 3

*Effect of adrenal steroids\* in protecting against circulatory failure following muscle trauma*

DATE	TIME	BLOOD PRES- SURE	PULSE	HEMO- GLOBIN	BLOOD SUGAR	REMARKS
Dog 9, 9.5 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone						
11/24	9:30 a.m.	mm. Hg 115	per minute 80	grams per cent 15.0	mgm. per cent 88	Trauma finished at 10:30 a.m. Pressure fell to 57 mm. Hg
	3:30 p.m.	104	184			
	10:30 p.m.	108	180	16.2	78	Strong, alert, ate food
11/25	10:30 a.m.	122	88	13.5	112	Leg still swollen but dog appears normal
Dog 10, 10.2 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone						
11/24	9:30 a.m.	108	88	14.9	88	Trauma finished at 12:30 p.m.
	5:30 p.m.	91	176			
11/25	11:30 p.m.	93	160	15.8	135	Active, ate food
	12:30 p.m.	103	116	11.5	116	Normal except for swollen leg
Dog 11, 9.8 kgm., 10 mgm. desoxycorticosterone acetate, after maintenance with cortical extract.						
1/15	10:00 a.m.	111	90	15.5	90	Trauma finished at 10:30 a.m.
	3:30 p.m.	116	148	15.8	92	
	5:30 p.m.	100	168	16.2	97	
1/16	10:30 p.m.	99	176	14.9	96	Good condition, ate food
	10:30 a.m.	116	120	14.0	92	Normal except for swollen leg
Dog 12, 10.0 kgm., 10 mgm. desoxycorticosterone acetate, after maintenance with desoxycorticosterone acetate						
12/12	9:30 a.m.	111	116	10.5	88	Trauma finished at 10:00 a.m.
	2:00 p.m.	48	176	11.0	67	Died at 2:30 p.m.
Dog 13, 8.6 kgm., 20 mgm. desoxycorticosterone acetate, after maintenance with desoxycorticosterone acetate						
1/7	10:15 a.m.	120	108	9.8	83	Completed trauma at 10:30 a.m.
	2:30 p.m.	40	200	10.2	53	Comatose. Died at 3:15 p.m.

\* Dogs 10 and 11 given four intravenous injections of 5 mgm. each at 2 hours before trauma, and at 2, 5, and 8 hours after; dogs 12 and 13 given two intramuscular injections at 12 and 2 hours before trauma; dog 14 given four intramuscular injections at 16, 12, 2 and 1 hour before trauma.

out inducing circulatory failure. The animals sustain an intense and prolonged vasoconstriction, the blood pressure remaining above normal throughout 80

per cent of the hemorrhage. During the last 20 per cent of the bleeding the pressure falls rapidly to shock levels. Blood dilution begins immediately and continues until the arterial pressure is restored to normal. The pressure rises spontaneously from shock levels, attaining normal within 24 to 48 hours.

In contrast, the blood pressure of the adrenalectomized dog receiving maintenance extract but no desoxycorticosterone falls steadily from the beginning of the hemorrhage. Removal of only 15 to 17 cc. blood per kgm. body weight suffices to reduce the pressure to shock levels. Upon cessation of the bleeding, the pressure may rise to about 60 mm. Hg, remain at this level for 3 to 6 hours, and then decline fairly rapidly to levels incompatible with life. There is no observable blood dilution during the course of the hemorrhage, although dilution may occur when the pressure reaches the low plateau of about 60 mm. Hg. However, it is never comparable in extent to that seen when liberal amounts of cortical extract or desoxycorticosterone are given previous to bleeding.

The dogs (previously maintained on extract) treated with 17-hydroxy-11-dehydrocorticosterone varied widely as to the total amount of blood which could be removed before the arterial pressure was reduced to 40–50 mm. Hg (table 4), but it totalled less than that which can be taken from the desoxycorticosterone treated animal. In several ways the response of the 17-hydroxy-11-dehydrocorticosterone-treated dogs was similar to non-primed animals receiving only maintenance doses of extract. 1, there was no initial pressure rise above normal; 2, blood dilution did not occur during the hemorrhage; 3, the arterial pressure rose spontaneously from shock levels to about 60 mm. Hg and was maintained at this level for several hours, during which interval blood dilution began. Unlike the untreated dog, however, the pressure then rose slowly but steadily toward normal. One of the three dogs tested (no. 14) failed to show a full pressure recovery by the 48th hour, when the experiment discontinued.

The hemorrhage experiments indicate that 17-hydroxy-11-dehydrocorticosterone is definitely less effective in protecting the adrenalectomized dog against circulatory failure following simple blood loss than is desoxycorticosterone, when the latter is used as a prophylactic on animals previously maintained on small doses of cortical extract. The dogs were not able to withstand the loss of as much blood, and the response to treatment was less dramatic. We have never observed a fall in blood sugar in the hemorrhaged adrenalectomized dog and it is very doubtful if marked alterations in blood sugar levels occur even when the animal is in collapse. Experiments now in progress do indicate, however, that even in hemorrhage the efficacy of desoxycorticosterone in protecting the circulation is reduced to zero when the animal has been maintained solely on this steroid previous to bleeding and hence is completely lacking in carbohydrate-active factors.

DISCUSSION. It seems more than coincidental that the circulatory failures most responsive to treatment with adrenal steroids having an oxygen at carbon-11 (carbohydrate active, 9–11) are at the same time those least affected by desoxycorticosterone (carbohydrate inactive 10–12) and are characterized by a significant decline in blood sugar. This suggests that the circulatory failure is



associated in some way with carbohydrate metabolism. The unexpected finding that desoxycorticosterone loses its protective effect upon the circulation unless the animal has been previously maintained on natural extract (presumably containing steroids with an oxygen at carbon-11) affords support for this general idea. The small daily maintenance doses of extract, although quite inadequate to prevent circulatory failure, apparently do serve to protect the animal against

TABLE 4

*Effect of adrenal steroids\* in protecting against circulatory failure following hemorrhage*

DATE	TIME	BLOOD PRESSURE	PULSE	HEMO- GLOBIN	BLOOD SUGAR	REMARKS
Dog 14, 9.7 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone. Removed 17.2 cc. blood per kgm. body weight						
12/1	10:00 a.m.	102	128	13.6	73	Completed hemorrhage
	11:10 a.m.	45	88	13.5		
	2:20 p.m.	67	148	12.0		
	10:20 p.m.	66	148	10.5	94	
12/2	11:00 a.m.	89	128	9.6	93	Given food, drank water
12/3	11:00 a.m.	79	144	9.0	93	Experiment discontinued
Dog 15, 8.5 kgm., 20 mgm. 17-hydroxy-11-dehydrocorticosterone. Removed 31.8 cc. blood per kgm. body weight						
12/1	10:00 a.m.	100	132	10.2	85	Completed hemorrhage
	1:05 p.m.	47	84	10.1		
	4:05 p.m.	62	136	10.1		
	10:05 p.m.	62	144	8.8	92	
12/2	10:05 a.m.	69	116	8.0	95	Given food, water
12/3	10:05 a.m.	94	100	6.7	90	Experiment discontinued
Dog. 16, 8.6 kgm. 20 mgm., 17-hydroxy-11-dehydrocorticosterone. Removed 20.0 cc. blood per kgm. body weight						
12/5	10:00 a.m.	122	72	13.3	83	Completed hemorrhage
	11:30 a.m.	47	98	13.3		
	2:30 p.m.	63	140	13.6	85	
	10:30 p.m.	62	100	12.2	85	
12/6	10:30 a.m.	101	96	11.5	84	Weak, given water Appears normal

\* Given in four intravenous injections of 5 mgm. each at 2 hours before the hemorrhage, and at 2, 5 and 8 hours after the hemorrhage.

drastic carbohydrate changes and thereby enable desoxycorticosterone to maintain the circulation.

The carbohydrate upsets involved are seemingly much more subtle than a mere decline in blood sugar level, which, except as to general trend, can not be correlated with blood pressure change. Various workers (7-9) have hinted that the intermediary metabolism of carbohydrate in the tissues themselves is ab-

normal in the adrenalectomized animal. It is tempting, therefore, to ascribe the fundamental disability, in so far as circulatory failure due to stress is concerned, to a breakdown of the carbohydrate cycle in the tissues. This metabolic dysfunction would be manifested by inability of the peripheral vasculature to perform properly, and eventuate in peripheral vascular failure and circulatory collapse.

The carbohydrate active steroids, when present in sufficient amounts, adequately protect the circulation; when present in quantities far too small to afford protection, nevertheless may be requisite for rendering the peripheral vessels responsive to desoxycorticosterone, which lacks the property of influencing carbohydrate metabolism.

The action of small doses of extract, ineffective in themselves, in rendering desoxycorticosterone active might also be accounted for on the assumption of a synergistic action between steroids in natural extract and desoxycorticosterone. Such a synergism can not be the sole explanation for circulatory failure following all stress procedures, however, for local blocking of the nerves by procaine, spinal anesthesia, or section of the spinal cord at the first thoracic vertebra,<sup>3</sup> enables desoxycorticosterone to become an effective agent for protecting the circulation following the single stage bilateral adrenalectomy. Spinal section also prevents the circulatory failure following intestinal stripping.<sup>3</sup> It seems necessary therefore to postulate the participation of a nervous factor in circulatory collapse resulting from these procedures. Nociceptive stimuli arising in the area of injury will, unless prevented by local or spinal anesthesia or spinal cord section, induce peripheral vascular failure, presumably by intense reflex stimulation of the vasomotor centers leading to eventual exhaustion, not of the centers themselves, but of the end organ, i.e., the peripheral vasculature.

The response of the vascular periphery to stimulation by barrages of impulses arising in the excited higher nervous centers would demand an increased metabolism of available carbohydrate. Since both the untreated and desoxycorticosterone-treated adrenalectomized dog lack essential factors for maintaining the normal cycle of carbohydrate metabolism the periphery would soon fail and circulatory collapse ensue.

Spinal section or anesthesia, by effectively blocking afferent impulses to the higher centers and so preventing excitation of these centers, should thereby prevent undue stress on the vascular periphery and, presumably, the depletion of carbohydrate reserves and early fatigue of the peripheral vasculature.

If the above interpretation should be found to be essentially correct, then it would appear that since other tissues of the body of the adrenalectomized dog participate in these disabilities of carbohydrate metabolism, the circulatory failure is but one manifestation of a generalized and fundamental derangement (13).

#### SUMMARY

1. The effectiveness of corticosterone and 17-hydroxy-11-dehydrocorticosterone in protecting the circulation of the adrenalectomized dog after stress

procedures has been tested, with emphasis on those types of circulatory failure which are not responsive to desoxycorticosterone therapy.

2. Unlike desoxycorticosterone, both corticosterone and 17-hydroxy-11-dehydrocorticosterone are effective after intestinal stripping and after the removal of both adrenal glands at a single stage operation. The action of corticosterone is inferior to that of 17-hydroxy-11-dehydrocorticosterone.

3. 17-hydroxy-11-dehydrocorticosterone is also effective after muscle trauma and hemorrhage, where desoxycorticosterone also shows activity. Its action in hemorrhage is qualitatively different and inferior to that of desoxycorticosterone.

4. Desoxycorticosterone fore treatment is ineffective in preventing circulatory collapse following muscle injury unless the animal has been receiving maintenance doses of cortical extract previous to trauma. The effectiveness of this steroid following hemorrhage is also greatly reduced or entirely lost unless very small amounts of carbohydrate-active material are present.

5. A possible explanation is suggested for the differences in action of adrenal steroids on the circulation based on 1, blood sugar changes which accompany the circulatory failure; 2, the rôle played by such procedures as spinal section, spinal anesthesia, etc., in protecting the circulation; and 3, the ineffectiveness of desoxycorticosterone when carbohydrate-active steroids are completely lacking.

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# THE EFFECT OF DEPLETION OF BODY POTASSIUM ON THE TIME OF SURVIVAL AFTER NEPHRECTOMY AND URETERAL LIGATION<sup>1</sup>

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Seventy-five years ago, evidence was brought out that potassium is a metabolite which, by accumulation in extracellular fluids, explains part of the toxic effects of complete abolition of renal function (1, 2). Recent work has again emphasized this point (3, 4, 5). The clearest demonstration is brought out by Hoff, Smith and Winkler (6) who showed that the toxic effects are accompanied by electrocardiographic changes which follow the same sequence as poisoning produced by the injection of potassium and each type of electrocardiographic alteration occurs at the same level of serum potassium in the two conditions. Miller and Darrow (7, 8) demonstrated that diets low in potassium made rats more resistant to the toxic effects of potassium. Injections of desoxycorticosterone acetate have a similar effect (9). The mechanism of this protective action is, in both cases, depletion of muscle potassium which enables this cation to enter the muscle when potassium is injected and thus protect extracellular fluids from a lethal rise in concentration.

The present work shows that the period of survival after nephrectomy or ureteral ligation is prolonged in rats subjected to procedures which have depleted body potassium before operation. The mechanism of this prolonged survival is delay in the post-operative rise in serum potassium which is made possible by low concentration of potassium in muscle.

**METHODS.** Bilateral nephrectomy and bilateral ureteral ligation were used to produce complete abolition of renal function. Ureteral ligation was found to be somewhat unreliable in rats because of the difficulty of being sure of complete obstruction. In the experiments reported, examination at autopsy indicated successful ligation. The nature of individual experiments will be made clear in the presentation of data.

The chemical methods are those applied to similar studies from the pediatric laboratory (7, 8). In the tables serum sodium and chloride are expressed in milliequivalents per liter of ultrafiltrate by use of a Donnan factor of 0.96 and the content of water in serum. The concentrations of potassium are given per liter of serum since data are not available which enable an accurate calculation of the concentration of potassium in the ultrafiltrate. Tissue analyses are calculated per 100 grams of fat-free solids.

The diets used were as follows: 1, Purina dog chow which contains 15 mM K

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per 100 grams, and 2, a synthetic diet containing 1.6 mM K per 100 grams (commercial casein 18, sucrose 25, dextrin 32, Crisco 22, yeast 2, salt mixture 4 parts).

*The survival of rats after abolition of renal function.* The length of each of the lines in figure 1 represents the period of survival of an individual rat. The experiments are divided into nine categories for which group 1 serves as the

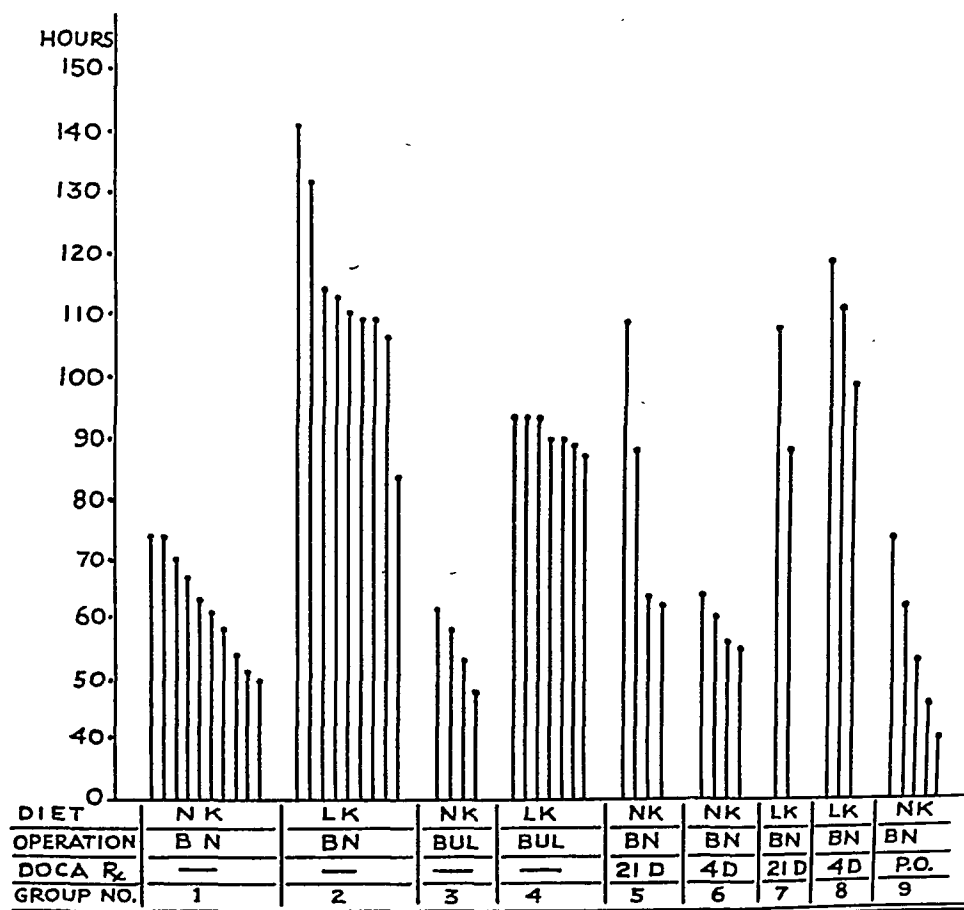


Fig. 1. Survival time after operation

NK = normal potassium diet; LK = low potassium diet; BN = bilateral nephrectomy; BUL = bilateral ureter ligation; 21D = 2 mgm. desoxycorticosterone acetate for 21 days before operation; 4D = 2 mgm. desoxycorticosterone acetate for 4 days before operation; P.O. = 2 mgm. desoxycorticosterone acetate every 24 hours after operation.

control for the nephrectomized rats and group 3 as the control for those subject to bilateral ureteral ligation.

Rats fed a diet of usual potassium content (group 1) survive bilateral nephrectomy for 50 to 74 hours, in this series none living longer than 74 hours. Rats fed a diet low in potassium for 21 days (group 2) survive bilateral nephrectomy for 84 to 141 hours. Since there is no overlapping between groups 1 and 2 the results are perfectly clear cut and indicate that depletion of body potassium by a

diet low in potassium enables rats to survive bilateral nephrectomy longer than rats with normal contents of potassium. These results are not vitiated by intake of potassium, since no food was taken after nephrectomy.

The results are equally clear for the effects of the diet on the period of survival after ureteral ligation (groups 3 and 4). As has been previously pointed out, dogs do not survive bilateral ureteral ligation as long as bilateral nephrectomy (10). The present experiments confirm this observation for rats. The chart shows that rats on a diet low in potassium survive longer than those on a normal diet. This difference is sufficient so that rats fed the diet low in potassium live longer after ureteral ligation than normal rats after nephrectomy. However, the rats on the diet low in potassium survive longer than normal rats when both groups are subjected to ureteral ligation.

The experiments using desoxycorticosterone acetate (groups 5 to 9) show that depletion of body potassium by injections of this compound increases the period of survival and this result is not an effect produced by action of the compound after nephrectomy. Two of four rats of group 5 which ate the normal diet but received 2 mgm. of desoxycorticosterone acetate daily for 21 days previous to operation survived longer than any rat of group 1. Group 3 originally contained six rats but two died at operation probably owing to cardiac lesions produced by prolonged injections of desoxycorticosterone acetate (11). The long survival of the two rats is sufficient to demonstrate the protective action of depletion of body potassium by injection of desoxycorticosterone acetate since it confirms previous observations (12). None of the four rats in group 6 which received 2 mgm. of the compound for 4 days before operation survived longer than 60 hours. Furthermore injection of the compound after operation in group 9 did not prolong the period of survival. The experiments in groups 6 and 9 were intended to bring out any immediate effect of desoxycorticosterone acetate which is independent of the depletion of body potassium. Groups 7 and 8 are supplements to groups 6 and 9 and show that desoxycorticosterone acetate does not alter the results produced by a diet low in potassium. Hence, although group 9 which received injections of desoxycorticosterone acetate after nephrectomy showed a short period of survival in two out of five instances, it is unlikely that this was due to a specific effect of the injection of the compound. Since unpublished data indicate that depletion of body potassium by daily injections of 2 to 4 mgm. of desoxycorticosterone acetate is not great for several days in rats, the contrast between group 5 and 6 shows that the depletion of body potassium rather than an immediate action on tissues is necessary for the protective action of desoxycorticosterone acetate.

*Serum and tissue electrolyte after abolition of renal function.* Table 1 shows the results of muscle and serum analyses. Certain rats marked with an asterisk were killed when their condition indicated that they would not live longer than one hour. The others were killed at the stated intervals and no exact estimate of the probable period of survival can be given. The table states into which group the various analyses would fit, although only the ones marked with an asterisk were used in estimating the period of survival. From other studies

average figures for serum and muscle are given for 1, normal rats fed a normal diet; 2, normal rats fed a diet low in potassium for three weeks, and 3, normal rats given 2 mgm. of desoxycorticosterone acetate daily for 21 days. In examining the figures from individual rats, changes are noted with reference to one of the three above control analyses of rats which have intact kidneys.

Rats previously fed a normal diet show a fairly rapid rise in the concentration of serum potassium after nephrectomy. The three moribund nephrectomized

TABLE 1

GROUP	NUMBER	DIET	P.O.	SERUM					MUSCLE PER 100 GM. FAT FREE SOLIDS					
				H <sub>2</sub> O	NPN	K	[Cl]	[Na]	H <sub>2</sub> O	Cl	Na	K	P	N
			hours	per cent	mgm. per cent	mM/l.	mM/l.	mM/l.	grams	mM	mM	mM	mM	grams
1*	41	NK	N69	93	510	11.9	111	155	354	13.7	13	50	34	14.8
1*	40	NK	N73	94	540	9.9	105	145	393	12.6	16	52	36	15.4
1*	103	NK	N74	94	400	11.5	99	135	343	9.0	10	51	34	14.4
2	31	LK	N42	94		4.7	99	142	345	6.1	14	39	33	15.1
2	29	LK	N43	92		4.9	114	167	313	6.8	15	40	35	15.5
2	30	LK	N90	94	580	5.8	97	156	328	7.9	15	40	33	16.3
2*	27	LK	N113	94	590	8.0	109	157	355	12.6	18	44	33	16.0
2*	28	LK	N114	94	680	5.2	103	151	367	11.8	15	48	35	16.9
3*	42	NK	U48	94		5.3	130	142	342	8.4	11	49	33	15.1
3*	44	NK	U61	93	420	8.1	101	145	376	8.4	11	52	34	16.3
6*	74	NK	N66	94	470	8.7	83	143	381	8.6	13	50	35	16.0
8	66	LK	N76	94	450	4.7	109	152	347	8.8	19	39	34	15.9
8	65	LK	N99	94	515	9.5	100	149	376	11.1	17	45	34	15.8
8*	64	LK	N118	93	505	7.1	105	152	410	16.2	25	39	31	15.9
9	101	NK	N74	94	515	11.4	105	143	393		16	50	33	15.9
9	75	NK	N46	94	396	16.7	101	147	405	12.8	15	51	35	16.6
9*	78	NK	N53	93	424	9.4	125	158	359	8.9	12	50	35	16.4
Control				93	30	4.0	113	147	341	7.2	10	49	33	15.8
Control				93	30	4.0	106	147	333	6.6	15	38	32	15.9
Control D.C.S.A.				93	30	3.7	108	152	328	6.3	15	40	32	15.8

\* Moribund.

P.O. hours after nephrectomy (N) or ureteral ligation (U) when rats were anesthetized, bled and killed.

[Cl] and [Na] indicate concentration per liter of ultrafiltrate of serum.

Diet is normal content of potassium (NK) or low in potassium (LK). The latter was given 21 days before operation.

rats, 40, 41 and 103, have values of 10 or more m. eq. per L. which is the concentration accompanied by serious electrocardiographic changes. The muscles of these rats show potassiums that have risen from the normal value of 48 to 50 or more m. eq. per 100 grams of fat free solids. If one assumes that the ratio of muscle chloride to the concentration of chloride in extracellular fluid measures approximately the volume of extracellular water, then normal rats have 2 to 3 m. eq. of non-extracellular sodium (probably intracellular). With the increase

in muscle potassium, evidence of non-extracellular sodium disappears. Of the two rats fed the normal diet and then subjected to ureteral ligation, rat 44 which was killed after 61 hours showed changes like those of the rats subjected to nephrectomy, while rat 42 shows normal serum and muscle potassium because the animal became moribund before the chemical changes had time fully to develop. Thus in normal rats, metabolism frees enough potassium to raise extracellular concentrations to toxic levels within 60 to 75 hours after abolition of renal function.

The rats which received a diet low in potassium (31, 29, 30, 27, 28) present a contrast, for rats 30, 27 and 28 have no or only slight elevation of serum potassium in spite of survival for 90, 113 and 114 hours. Only one of these animals (28) had the usual level of muscle potassium while those of rats 29, 30 and 31 were still abnormally low and that of rat 27, low normal. Muscle chloride is high in the two moribund rats (27 and 28) with normal muscle potassiums. In these animals the muscle sodium gives no evidence of abnormally high intracellular sodium. In the remainder of the rats of group 2, muscle chloride is normal but muscle sodium is high, showing an excess of intracellular sodium.

The nephrectomized rat 74 which had been on the normal diet and had received 2 mgm. of desoxycorticosterone acetate for 4 days shows muscle and serum analyses essentially like uninjected rats. Those receiving desoxycorticosterone acetate after nephrectomy (75, 78 and 101) do not differ from simple nephrectomized rats except perhaps rat 75. This rat became extremely sick within 15 minutes after a subcutaneous injection of the synthetic compound. The explanation of the sudden collapse is the high value for serum potassium (16 mM per L.). Since this result was not a regular occurrence, it does not represent a specific action of desoxycorticosterone acetate.

**Discussion.** Obviously potassium poisoning is only one of the modes of exit after nephrectomy or ureteral ligation. It is interesting that by delaying the onset of the rise in concentration of potassium in serum, the period of survival is almost doubled. Histological studies show no lesion in the other tissues after nephrectomy. However, after ureteral ligation, foci of necrosis in cardiac and smooth muscle can be demonstrated. Since there is no difference in the various groups in this respect, the rise in concentration of serum potassium does not play an important rôle in the production of these lesions. The animals receiving desoxycorticosterone acetate, particularly if on a diet low in potassium, show extensive areas of necrosis in the heart muscle and replacement by fibrous tissue. These lesions are similar to those seen in rats fed a diet low in potassium (13, 14, 15) or those receiving large doses of desoxycorticosterone acetate (11) and have no relation to abolition of renal function. The rats on a diet low in potassium or receiving the synthetic hormone had large kidneys (19). Thus the diet low in potassium or the injection of desoxycorticosterone acetate produces certain changes in the heart and kidneys but the effect of these procedures does not alter or prevent histological lesions produced by ureteral ligation. The increased survival is apparently entirely connected with the chemical and functional changes accompanying the rise in concentration of potassium in serum.



The mechanism of the protective action of the diets low in potassium and injections of desoxycorticosterone acetate is relatively simple. It is the same as the protective action against injections of KCl which may be produced by depletion of muscle potassium (7, 8). Briefly, our previous studies have indicated that normal muscle potassium tends to vary between 44 and 49 mM per 100 grams of fat free solids. When it drops below this figure, serum potassium tends to be abnormally low, and when it is above 50, serum potassium is abnormally high. In normal muscle there are apparently 2 to 3 mM of intracellular sodium per 100 grams of fat free solids. As muscle potassium increases, this intracellular sodium disappears; as muscle potassium reaches low figures, intracellular sodium increases. By various means we have been unable to increase muscle potassium beyond 53 mM per 100 grams, since high values were accompanied by concentrations of potassium in serum which produced heart block. The ceiling, however, may actually be related to the amount of intracellular sodium which potassium may displace. At least, rats with high intracellular sodium and low potassium can receive larger injections of KCl before there is a fatal rise in the concentration of potassium in serum. Since the nephrectomized rats do not eat, consumption of body stores frees potassium and the ability of the muscle to absorb potassium is the chief protection against a lethal concentration of potassium in serum. In rats fed a normal diet, the amount of potassium which the muscles can absorb is so small that potassium poisoning is usually the limiting factor in survival after nephrectomy. In rats previously fed a diet low in potassium or those treated preoperatively with desoxycorticosterone acetate, the amount of potassium which may be taken up by the muscle is sufficiently large so that survival is longer and potassium poisoning only occasionally develops.

Selye and Nielson (12) showed that the injection of 10 mgm. of desoxycorticosterone for four days before nephrectomy increased the period of survival. In our experiments 2 mgm. over a similar period was ineffective. There is other evidence that the negative balance of potassium is increased by large doses of the synthetic hormone over that obtained by smaller doses (17, 18). The doses which we used have been shown to reduce muscle potassium to 38 to 42 mM per 100 grams of fat free solids in ten days and about 35 in 21 days.

The serum sodiums and chlorides give some evidence of loss of intracellular sodium from muscles since, in most cases, the concentration of chloride in serum ultrafiltrate decreases while the concentration of sodium remains relatively constant. However, there is only slight evidence that this difference is more marked in the rats fed the diet low in potassium than in the rats on the normal diet.

The rise in non-protein nitrogen is the same in all groups, so depletion of body potassium does not affect this phase of experimental uremia.

Since Hoff, Smith and Winkler (6) pointed out that fatal rise in serum potassium is unlikely in clinical uremia, the present studies have little direct bearing on the problems of nephritis. Specifically diets low in potassium would not be expected to prolong the life of a nephritic patient. However, administration of large doses of potassium salts as a diuretic might be dangerous in certain patients.

## SUMMARY

Rats depleted of body potassium by a diet low in potassium or by preoperative injection of desoxycorticosterone acetate survive nephrectomy or ureteral ligation longer than rats on a normal diet. This phenomenon is accompanied by a delay in the rise of serum potassium to toxic levels. This delay in rise of serum potassium is explained by an increase in muscle potassium from abnormally low values towards normal values.

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# EFFECTS OF THE REMOVAL OF THE ANTERIOR LOBE OF THE HYPOPHYSIS ON SOME RENAL FUNCTIONS

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We have reported (1, 2) that diodrast<sup>2</sup> (D) and inulin plasma clearances and maximum tubular output of diodrast ( $DT_m$ ) are decreased by hypophysectomy, diodrast and inulin extractions are unchanged. The present paper extends these observations. We are here reporting on 12 dogs; of these 6 had total hypophysectomies (removal or destruction of all of hypophysis, including stalk and median eminence), 4 had simple hypophysectomies (section of stalk with removal of dependent gland), and 2 which showed diabetes insipidus, had been rendered so by appropriate hypophthalamic lesions without any direct operation on the hypophysis. All operations were carried out through the oral approach. In half of the animals, which included representatives of all the classes, the lesions were checked by examination of serially cut sections of the hypothalamus and sella turcica with its contents. The effects of the operative procedures on D and inulin plasma clearances and renal extractions, on renal plasma flow (RPF), on  $DT_m$ , on blood volume and on the patency of glomeruli will be considered.

**PROCEDURE.** Trained female dogs in the postabsorptive state were used. They were on a diet of 1 pound of raw horsemeat daily with Purina dog chow ad lib. Plasma inulin levels of 8 to 14 mgm. per cent were attained by giving 0.34 gram of inulin per kilo as a 25 per cent solution in saline subcutaneously about 90 minutes before beginning the experiments; with hypophysectomized dogs showing lower than normal clearance, about two-thirds of this dose is given. Plasma D iodine levels of 1 to 2 mgm. per cent were attained by giving 0.25 cc. of D solution (35 per cent) per kilo subcutaneously (diluted 1 to 3 with saline) about 45 minutes before an experiment.

Immediately following D administration, 2.5 per cent body weight of water was given by stomach tube. Plasma D levels suitable for  $DT_m$  determinations were attained by giving an intravenous priming dose of 0.66 cc. of D per kilo, followed by a sustaining injection of 2 cc. per minute of solution containing 0.5 cc. D solution per kilo per 100 cc. with saline as diluent; with hypophysectomized dogs the sustained dose is 60 per cent of the normal. Sustaining infusion is carried out for 25 minutes before first urine collection is begun; in most clearance experiments 3 or more urine collections were made, 2 or more collections being made in the  $T_m$  experiments. The urine collection periods were 20 to 30 minutes; the bladder was washed twice with 30 cc. of saline and urine plus

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<sup>2</sup> The diodrast for this investigation was supplied through the courtesy of the Winthrop Chemical Company.

washings were made up to 250 cc. Mean plasma levels were estimated from curves through 3 or more observed points; with subcutaneous administration fairly constant plasma levels and quite smooth curves are obtained. *D* was determined by the method of White and Rolf (3). Inulin was determined by a modification of Corcoran and Page's method (4) (the readings are made in photoelectric colorimeter at a volume of 7 cc.). One centimeter of filtrate and 3 cc. of diphenylamine solution (7.5 cc. of 20 per cent diphenylamine in 95 per cent ethyl alcohol plus 56 cc. of ethyl alcohol plus 62.5 cc. of concentrated hydrochloric acid) are put into a boiling water bath for exactly 15 minutes. The

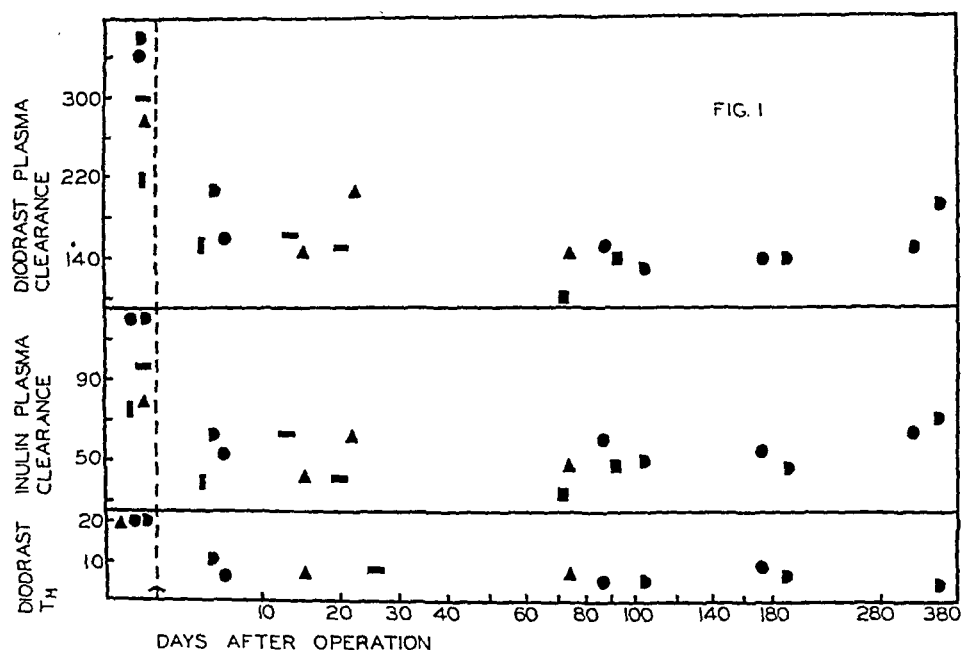


Fig. 1. Plots of  $DT_m$  in mgm. per min. per sq. M., inulin and *D* plasma clearances, cc. per min. per sq. M. before and after operation for completely hypophysectomized dogs. Each point on the clearance plots represents an average of 3 consecutive 20 to 25 minute periods, each point on the  $T_m$ 's the average of 2 or more consecutive 20 minute periods. The surface areas for all charts were calculated on the basis of the dogs' original weights because it was assumed that no change in kidney's weight would accompany the obesity that developed in the animals because of the nature of their hypothalamic injuries.

tubes are quickly cooled in ice water and made up to 7 cc. with 95 per cent alcohol. Great care is taken in calibration of these tubes. The blue solution is transferred into another test tube for reading, all readings being made in the same test tube by means of a photoelectric colorimeter.

**RESULTS.** *D* plasma clearance. The results are shown in figures 1, 2 and 3. It is seen that after loss of anterior lobe the clearance falls, being definitely lowered by the 5th to 7th postoperative day on both the complete and simple hypophysectomy dogs (figs. 1 and 2); this fall persists apparently permanently. The values after operation are about half the normal. The average of 45 periods taken on or after the 5th postoperative day with 5 complete hypophysectomy

dogs shows a D plasma clearance value of 155 cc./minutes/ $M^2$ , as compared with a normal average of 237 on the same dogs. That the fall is due to loss of the anterior lobe is shown by the findings that *a*, the results are essentially the same after complete hypophysectomy as after the simple, where part of the *pars nervosa* remains, and *b*, there is no fall on destruction of the *pars nervosa* only (fig. 3), except that there may be a transitory rise followed by a transitory fall with a return to a persistent normal.

The fall in D clearance after loss of the anterior lobe begins too early to be ascribed to a fall in metabolic rate known to occur in 6 to 8 weeks in hypophysectomized animals. That there is at first an actual deficit of renal blood flow with respect to the body's needs is further indicated by the finding, based on a limited number of observations, that the plasma urea level rises and is elevated for a few weeks after which it returns toward normal without any increase in D clearance.

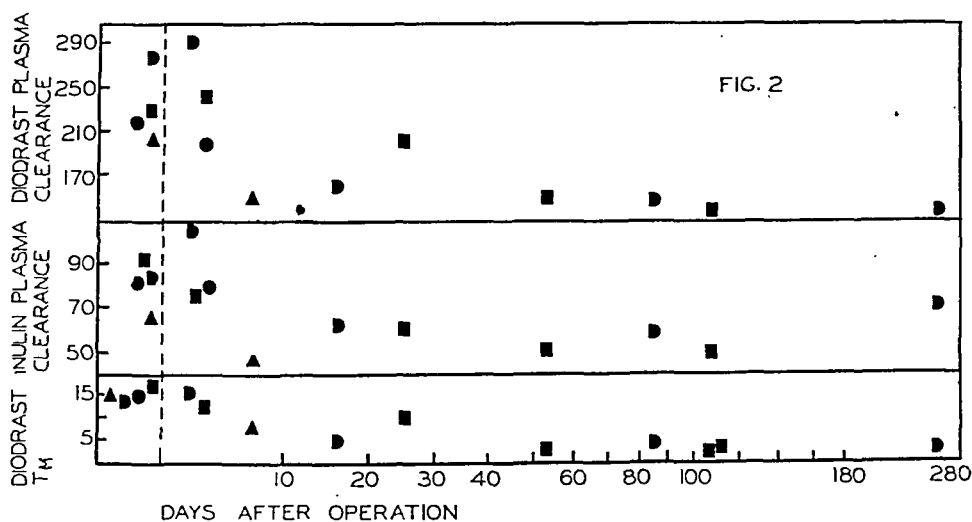


Fig. 2. Plots of  $DT_m$  in mgm. per min. per sq.  $M$ ., inulin and D plasma clearances, cc. per min. per sq.  $M$ . before and after operation for simply hypophysectomized dogs. Each point on the clearance plots represents an average of 3 consecutive 20 to 25 minute periods, each point on the  $T_m$ 's the average of 2 or more consecutive 20 minute periods.

This may mean that as the nitrogen metabolism diminishes several weeks after the operation, a previously inadequate renal blood flow becomes adequate.

Neither simple nor complete hypophysectomy has any effect on the renal extraction of D nor the tubular extraction of D at plasma levels suitable for clearance determinations—White, Heinbecker and Rolf (2). We have no observations on D extractions by diabetes insipidus dogs with intact anterior lobes, but since neither D plasma clearance nor  $DT_m$  is changed here and since there is no difference in extractions with simple and complete hypophysectomy, there is no reason to suppose that the *pars nervosa* has any effect on D extractions.

*RPF*. Since the extraction of D is not affected by hypophysectomy, the expression  $D \text{ plasma clearance} / D \text{ plasma extraction} \times 1.2$  (White, Heinbecker and Roff (2), White and Heinbecker (1)) is equally applicable to normal and to hypophysectomized dogs. This is found to give good agreement with the

inulin plasma clearance/inulin plasma extraction. Since it has been established by White and Heinbecker (1) that D plasma clearance  $\times 1.2$  shows satisfactory agreement with simultaneously determined RPF, provided extraction is normal, it is possible to estimate RPF on hypophysectomized as well as normal dogs without obtaining renal vein blood.

*Inulin clearance.* After complete or simple hypophysectomy the inulin clearance falls within a few days and remains permanently low (figs. 1 and 2). The average of 45 periods taken on or after the 5th postoperative day with 5 hypophysectomized dogs shows an inulin plasma clearance value of 54.2 cc./minute/ $M^2$ , as compared with a normal average of 104 on the same dogs. The

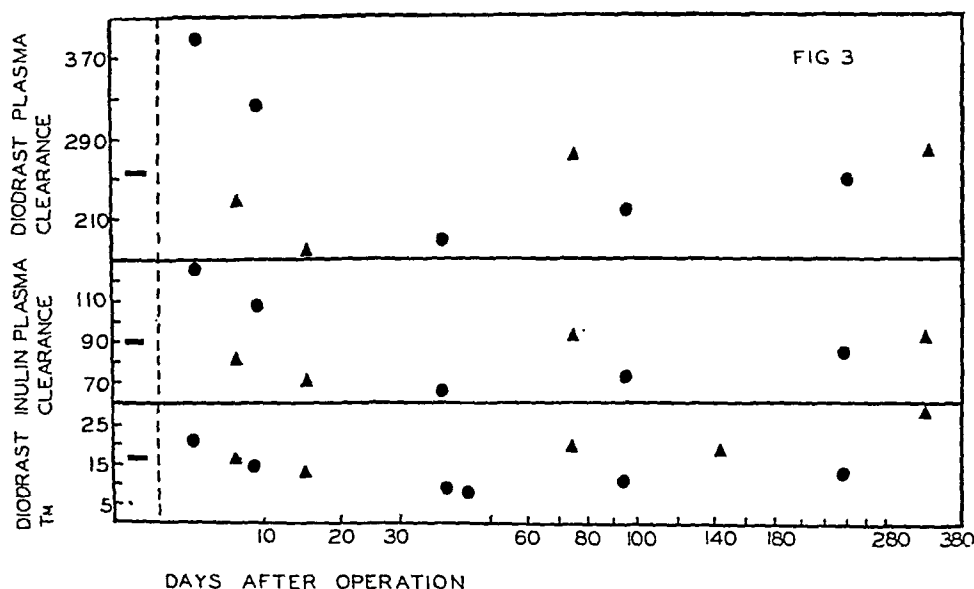


Fig. 3. Plots of  $DT_m$  in mgm. per min. per sq. M., inulin and D clearances, cc. per min. per sq. M. before and after operation for dogs with the anterior lobe in, the pitressin forming tissue functionless. Each point on the clearance plots represents the average of 3 consecutive 20 to 25 minute periods, each point on the  $T_m$  plot represents the average of 2 or more 20 minute periods. The points for the normals represent the average of all values for our experimental dogs.

average of 21 periods taken on or after the 7th postoperative day with 3 simple hypophysectomy dogs shows an inulin plasma clearance of 54 cc./minute/ $M^2$ , with a normal average of 80 on the same dogs. The filtration fraction is not significantly changed by either type of hypophysectomy. We do not believe that there is a significant difference between the effects of simple and complete hypophysectomy on the D and inulin clearances.

The dogs in which diabetes insipidus was produced by hypothalamic puncture without operative injury to the anterior lobe show a slight fall in unulin clearance, with a return to normal; there may be an early rise preceding the fall. In general, the present findings agree with our earlier report (5) on creatinine clearances in diabetes insipidus dogs.

*Diodrast  $T_m$ .* After complete or simple hypophysectomy  $DT_m$  falls rapidly; within a few days it is about half the normal, with a subsequent further fall (figs. 1 and 2). The average of 26 periods on or after the 5th postoperative day with 4 complete hypophysectomy dogs gives a  $DT_m$  value of 5.5 mgm./minute/ $M^2$ , where the normal had been 21 on the same dogs. The average of 16 periods on or after the 7th postoperative day with 3 simple hypophysectomy dogs shows a  $DT_m$  figure of 4.5 mgm./minute/ $M^2$ , where the normal had been 15.4 on these dogs. There is no tendency to return toward normal. There is no permanent effect on  $DT_m$  in the diabetes insipidus dogs with intact anterior lobes (fig. 3). There may be some initial fall but there is a return to normal within a few weeks.

*Blood volume.* The effects on blood volume of the loss of the glandular hypophysis, of all pitressin forming tissue and of both together were determined by

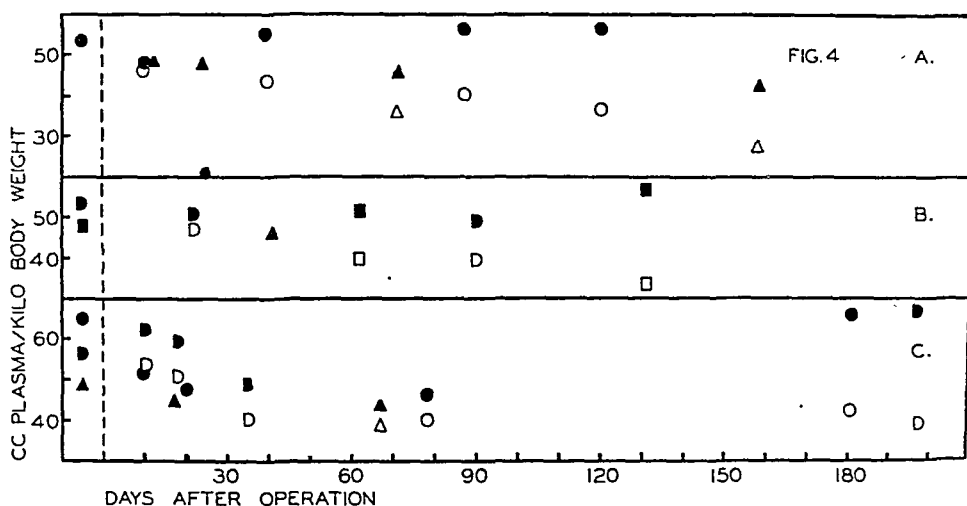


Fig. 4. Plots of plasma volume per kilogram body weight of (A) 2 dogs with intact anterior lobes, pitressin forming tissue functionless, (B) 2 simply hypophysectomized dogs, (C) 3 completely hypophysectomized dogs. Solid marks represent plasma volumes calculated on the basis for stabilized original weights, open marks represent plasma volumes calculated on the basis for actual weights at time of observations.

the blue azo dye (T-1824) method as described by Gibson and Evelyn (6). The values are given as cubic centimeters of plasma per kilo body weight. Because many of the animals gained weight during the period of observation the plasma volumes were calculated on the basis of the original stabilized normal weights and on the actual weights of the animals at the time after operation at which the observations were made.

Analysis of the results (fig. 4) indicates that the blood volume calculated on the basis of the original weights of the animals is unchanged or slightly increased by loss of the glandular or neural hypophysis. Calculated on the basis of the actual body weights of the animals at the time of the observations there is a diminution of blood volume, the degree of which in general varies from 20 to 80 per cent of the percentage increase in body weight.

The amount of decrease in blood volume calculated on the basis of the actual weight at the time of the observation is not more than 5 to 10 per cent until 6 to

8 weeks have elapsed since the removal of the anterior lobe. The depression of D and inulin clearances and particularly of  $DT_m$  is fully developed and stabilized at a low level well before a decrease in blood volume has developed. It follows that the decrease in renal function following anterior lobe loss is not to be attributed to a decrease in blood volume.

*Glomerular intermittence.* In 2 totally and in 2 simply hypophysectomized dogs the phenomenon of glomerular intermittence was looked for according to the method outlined by White (7). Examination of many sections, two of which are shown in figure 5, failed to reveal evidence of any glomerular intermittence. The diminished renal function exhibited by animals deprived of their glandular hypophysis apparently cannot be attributed to glomerular intermittence.

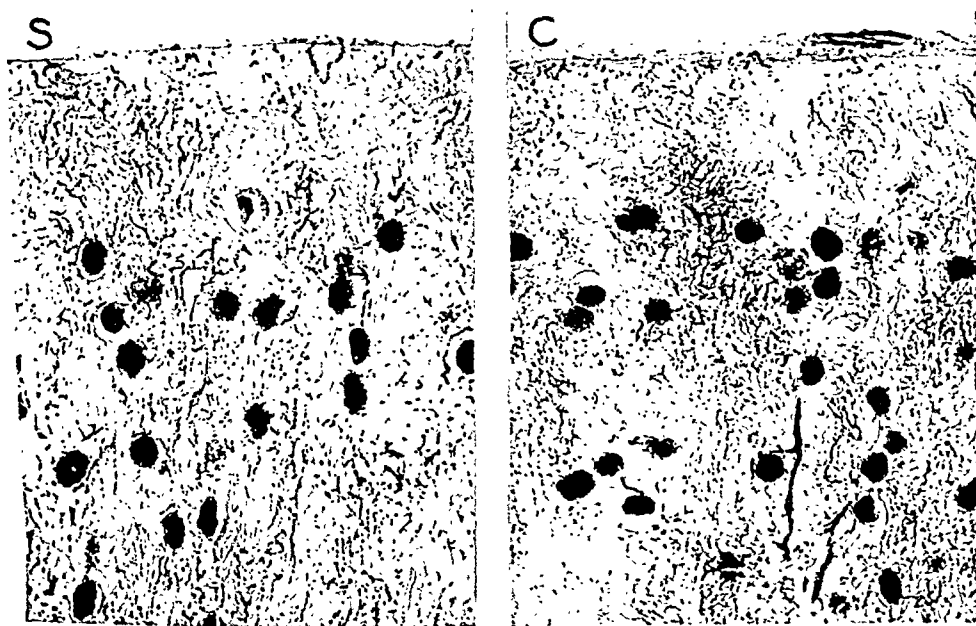


Fig. 5. Photomicrographs 45 diameters showing kidney glomeruli in dogs after ink injection. S—after simple hypophysectomy. C—after complete hypophysectomy. Note all glomeruli are well injected.

*Blood pressure determinations.* Blood pressure readings made by the auscultatory method on 4 simple and 4 total hypophysectomy dogs have shown that there is no increase and frequently a 10 to 15 per cent decrease in systolic and diastolic pressures four to six months after operations. This is of interest in view of the 50 per cent diminution in renal blood flow shown to be present in these two classes of animals.

**DISCUSSION.** The results demonstrate that the anterior lobe of the hypophysis plays an important rôle in the maintenance of normal renal blood flow. Its loss results in an average decrease in blood flow of approximately 50 per cent in 4 to 6 days and this reduction has been maintained throughout the period of our observations of 9 to 12 months. The quick onset of the change proves that the result is not secondary to thyroid or adrenal cortical atrophy.

In our experiments exact determinations have been made only of renal blood



flow. Roentgenograms of the hearts of four dogs showing diabetes insipidus, two with, and the others without, the adeno hypophysis show definitely in the latter evidence of a decrease in amplitude of the cardiac contractions and in cardiac output and hence in general blood flow. It is doubted that this method in dogs is sufficiently quantitative for accurate measurements. However, some decrease in general blood flow may be assumed because of the decrease in basal metabolic rate known to follow removal of the adeno hypophysis. This decrease has been shown to be about 16 per cent in dogs by Houssay et al. (8). It comes on gradually in 6 to 8 weeks. Support of evidence of a decrease in general blood flow by Aschner (9) in showing that in hypophysectomized dogs subcutaneously administered epinephrine has little effect on blood sugar whereas when administered intravenously, Russel and Cori (10) or intraperitoneally, Heinbecker and Weichselbaum (11), it has a normal influence. On the other hand the decrease in renal blood flow is established at nearly its maximum level of 50 per cent within a week. It appears then that the depression in renal blood flow is essentially an expression of a loss of a specific influence on the kidney by the adeno hypophysis. Also it has been observed by us that in the dog deprived of *pars distalis*, the quantity of intravenously administered nembutal required to produce comparable anesthesia is approximately half that required by the normal.

The decrease in renal functions evidenced through depression of the inulin and diodrast plasma clearances is attributed to the diminished renal blood flow. The relatively greater decrease in the  $DT_m$  cannot be attributed solely to the decrease in blood flow but must reflect an actual decrease in the capacity of the tubules to transport diodrast at high plasma levels.

The interesting observation that in 2 or 3 days following manipulation of the anterior lobe of the hypophysis there may occur an actual increase in inulin and D clearances and in  $DT_m$  suggest that extracts of the anterior lobe may possibly be used to stimulate renal functions. This subject is at present under investigation.

#### SUMMARY

Renal function studies on 12 dogs are reported. Six of the dogs had a total hypophysectomy, 4 a simple hypophysectomy and 2 denervation of the entire neurohypophysis with preservation of the anterior lobe.

D and inulin plasma clearances and  $DT_m$  were determined in all dogs before and at intervals after operation for 9 months to a year. In some of the animals D and inulin extractions were determined on explanted kidneys.

D and inulin plasma clearances and  $DT_m$  are markedly decreased by loss of the anterior lobe of the hypophysis. D and inulin extractions at low plasma levels are unaffected. Loss of the pitressin forming tissue does not affect these renal functions.

The decrease in D and inulin plasma clearances is attributed to a decrease in renal plasma flow. The decrease in  $DT_m$  is due not only to the decrease in renal blood flow but also to a depression of the functional capacity of the tubules to transport D at high plasma levels.

Loss of the anterior lobe of the hypophysis or loss of the entire hypophysis does not result in a diminution of blood volume when the calculations are made on the basis of the original weights of the animals. If the dogs become obese because of the nature of the operation to which they were subjected the blood volume calculated on the basis of actual weight at the time of the observation shows a decrease averaging 50 per cent of the percentage increase in body weight.

Loss of the anterior lobe of the hypophysis does not result in glomerular intermittence.

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# RELATION OF THE ADENO AND THE NEUROHYPOPHYSIS TO INSULIN SENSITIVITY AND SUGAR TOLERANCE IN THE DOG

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Geiling, Campbell and Ishikawa (1) reported that posterior lobe extracts protected hypophysectomized dogs against the hypoglycemic action of insulin. They reported also that removal of the anterior lobe did not render the dogs insulin sensitive. Houssay and Potik (2) attributed insulin sensitivity in the toad to loss of the anterior lobe and they showed that transplants of anterior lobe protected against insulin hypersensitivity. Pencharz, Cori and Russel (3) reported that removal of the posterior lobe of the hypophysis with only slight injury to the anterior lobe did not increase the sensitivity of rats to the convulsive action of insulin. Removal of the anterior lobe only, leaving the posterior lobe intact, resulted in a marked increase in insulin sensitivity, equal to that observed after complete hypophysectomy. The conclusions of Houssay and Potik in the toad and of Pencharz, Cori and Russel in the rat generally are assumed to apply throughout the animal kingdom in spite of the contradictory evidence of Geiling, Campbell and Ishikawa for the dog.

Operative procedures involving the hypophysis of a type permitting the functional study of its glandular and neural divisions separately were carried out on dogs to investigate their insulin sensitivity and glucose tolerance to orally and intravenously administered glucose. The operated dogs fell into three classes: 1, those "simply" hypophysectomized, i.e., removal of the anterior and posterior lobes; 2, those completely hypophysectomized, i.e., removal of the entire adeno and neurohypophysis (median eminence included); and 3, puncture dogs, those in which by appropriate puncture the entire neurohypophysis was rendered functionless with minimal injury to the anterior lobe. The inference that the anterior lobe functions normally is based on the demonstration of a normal renal blood flow. It has been shown that in such animals loss of the anterior lobe decreases renal blood flow in the dog by 50 per cent (White and Heinbecker, 4).

Two animals of classes 2 and 3, one simple hypophysectomy and one normal dog are reported on. The operations on these animals had been carried out 9 to 12 months previously. Many other simple hypophysectomy and normal dogs have been previously reported on with results similar to those herein given (Weichselbaum, Heinbecker and Somogyi, 5; Heinbecker, Somogyi and Weichselbaum, 6). The insulin and glucose were administered in the fasting state; the amounts together with the route of administration are indicated in the legends of the figures. True sugar values were determined according to the Shaffer-Somogyi method. The animals had been kept on a diet of Purina chow and

<sup>1</sup> Recipient of a grant in-aid-of-research by the Commonwealth Fund.

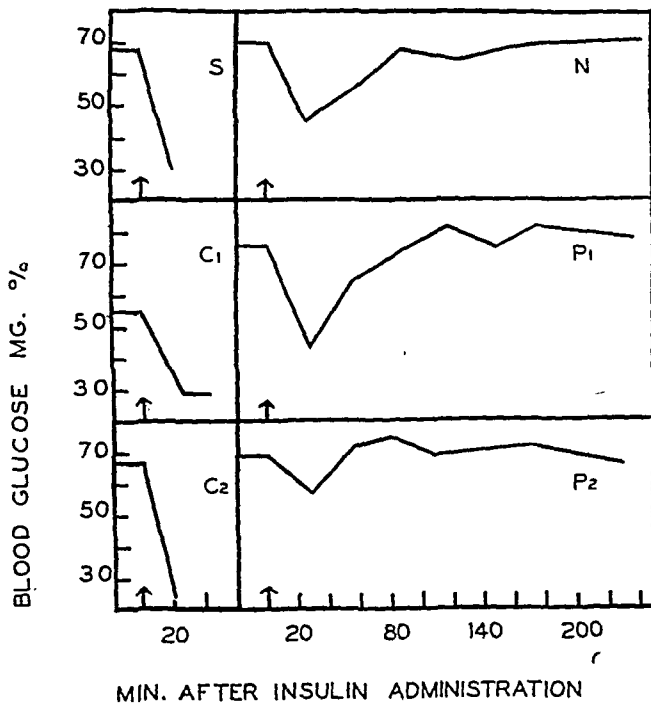


Fig. 1. Insulin response in normal, *N*, simple hypophysectomy, *S*, complete hypophysectomy, *C*, and puncture *P* dogs; 0.125 unit insulin per kilo given intravenously. In all animals without the adenohypophysis hypoglycemic manifestations developed necessitating discontinuance of the experiment and the administration of glucose.

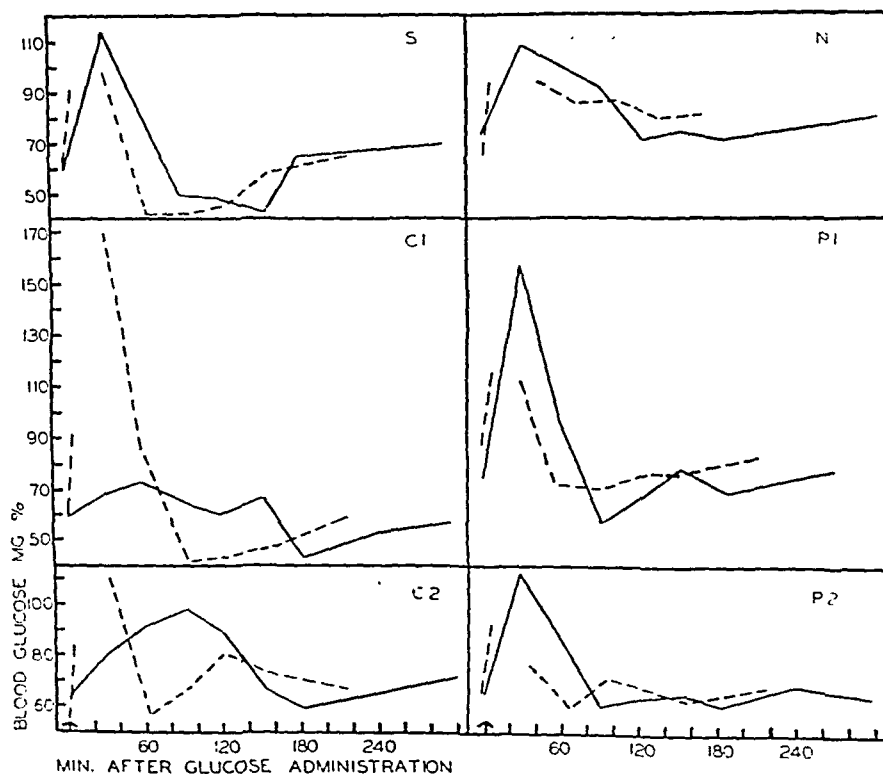


Fig. 2. Glucose tolerance curves in normal, simple hypophysectomy, complete hypophysectomy and puncture dogs after the oral and intravenous administration of glucose. Oral dose 0.85 gram per kilo, intravenous dose 1.5 grams per kilo, — after oral, ---- after intravenous, administration.

horse meat. The dosages of insulin and glucose were calculated on the basis of the original stabilized weight because the totally hypophysectomized and "puncture" dogs had become quite obese (80 per cent weight increase on an average), the simply hypophysectomized dogs had gained approximately 20 per cent.

Analysis of our results (figs. 1 and 2) indicates that dogs with the adeno-hypophysis removed tend to show a lower fasting blood sugar level than the normal.

Insulin sensitivity is shown to be due entirely to a loss of the adeno-hypophysis. The degree of this sensitivity is so great that even after the use of a small testing dose, 0.125 unit per kilo, the experiments on dogs deprived of their adeno-hypophysis had to be terminated within 30 minutes because of the development of salivation, rapid respiration, stupor and a loss of ability to stand.

No definite effect on sugar tolerance of the loss of the adeno-hypophysis or neurohypophysis alone is demonstrable by means of the blood sugar response to orally or intravenously administered glucose. Dogs deprived of one or both divisions of the hypophysis show a measure of instability of the blood sugar level in that the degree of hypoglycemia which even normally may follow hyperglycemia is exaggerated. In the completely hypophysectomized dogs the glucose curves following oral administration tend to be flat. This might reasonably be attributed to a slowness of absorption since a diminution in blood flow has been shown to exist in such animals (White, Heinbecker and Rolf, 7). However, this flatness is not shown in the simply hypophysectomized animals which have a similarly decreased blood flow.

#### SUMMARY

The fasting blood sugar of simple and of total hypophysectomy dogs is lower than in the normal.

Insulin sensitivity in such dogs is due entirely to a loss of the adeno-hypophysis.

No definite effect on sugar tolerance of the loss of the adeno or neurohypophysis is demonstrable by means of the blood sugar response to orally or intravenously administered glucose. Loss of one or both divisions of the hypophysis results in an exaggeration of the degree of hypoglycemia which even normally may follow hyperglycemia.

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## PRODUCTION OF INSULIN RESISTANCE IN DEPANCREATIZED DOGS

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Infection is the most common cause for insulin resistance in diabetes mellitus, but the mechanism is not understood. Of the several theories advanced to explain this resistance, that of decreased insulin secretion by the pancreas appears to have been more generally accepted. Soskin and co-workers (1) were able to produce insulin resistance in depancreatized dogs, but their animals died during or immediately after the acute experiment. Their results have been criticized because they may have been due to the so-called terminal affair. The purpose of this investigation is to ascertain whether or not insulin resistance can be produced in depancreatized dogs in chronic experiments.

**METHOD.** Depancreatized dogs were placed in metabolism cages and fed a mixture of ground lean beef muscle, cane sugar, dried pancreas and bone meal. The 24 hour urine excretion was collected and analysed quantitatively for dextrose. The animals were catheterized at the end of each 24 hour period to insure accurate collections. Insulin was administered just before the two daily feedings and in amounts sufficient to make the animals utilize most of the ingested sugar. After the diabetes became stabilized foreign proteins, cultures of bacteria or locally irritating substances were injected subcutaneously, intramuscularly, or intravenously. Typhoid vaccine and typhoid H antigen were administered in doses of from 5 million to 1 billion organisms in single or in daily doses. Skimmed milk was administered in doses of from 5 to 20 cc. in single or in daily injections. Cultures of *Bacillus coli* were administered intravenously in doses of from 1 to 5 cc. in single injections. Turpentine was injected subcutaneously in single doses of 1 cc. Blood sugar estimations were obtained occasionally in each animal. The nitrogen excretion was studied during several observations. Most of the animals were used for several periods of observation and these periods varied from 12 to 66 days. There were 23 periods of observation on 7 dogs. Two of the dogs were sacrificed at the height of the insulin resistance and complete post-mortem studies were made. Particular attention was directed to the liver and all endocrine glands. All animals were sacrificed at the end of the studies and careful search was made for any pancreatic tissue. Small pieces of pancreas were left at the time of the pancreatectomy in two animals and these were found during the post-mortem examination.

**RESULTS.** Insulin resistance was produced in varying degrees in 16 of the 23 observations. The number of observations on each animal, the number showing no insulin resistance and the number showing different degrees of insulin resistance are shown in table 1. It is to be noted that only one animal (dog 1)

failed to show any insulin resistance, whereas three of them (dogs 4, 5 and 6) showed some resistance during each period of observation. One animal (dog 4) developed spontaneously an extreme insulin resistance which lasted for 30 days. Later a moderate resistance developed following administration of a foreign protein. The potential dextrose intake and the insulin dosage were kept constant in most instances and the degree of insulin resistance was ascertained by the increase in urinary dextrose. An increase of 10 grams or less of urinary dextrose daily was classified as no insulin resistance, an increase of from 10 to 35 grams was considered to be slight, an increase of 35 to 65 grams daily was classified as moderate resistance and an increase of 65 grams or more was considered to be extreme insulin resistance. The estimated blood sugars increased during the periods of insulin resistance and declined as the resistance subsided. The data obtained during the production of moderate insulin resistance in one animal is shown in table 2. It is to be noted that the urinary dextrose increased from

TABLE 1

DOG	EXPERIMENT NO.	INSULIN RESISTANCE			
		None no.	Slight no.	Moderate no.	Extreme no.
1	3	3	—	—	—
2	4	2	1	1	—
3	2	1	—	—	1
4	1	—	—	1	—
5	2	—	2	—	—
6	5	—	2	3	—
7	6	1	—	5	—
Total.....	23	7	5	10	1

Table shows the number of experiments on each dog, the number showing no insulin resistance and the number showing different degrees of insulin resistance.

an average of 9 grams daily to 71.5 grams daily. There was an accompanying increase in urinary volume and not shown in the table an increased urinary nitrogen. An illustration of extreme insulin resistance is shown in table 3. The data presented from the 3rd of March to the 16th day of May are averages for 3 to 7 day periods. After the insulin dosage reached 80 units daily the injections were made 4 times daily instead of the usual 2 times. The animal was sacrificed on the 17th of May and histological studies of the liver, pituitary, thyroid, and adrenals revealed no significant changes.

An analysis of the effectiveness of the different agents employed in production of insulin resistance is of interest. It will be noted in table 4 that typhoid vaccine does not appear to be as effective as typhoid H antigen. This may be due in part to the fact that the former was employed early in the studies and smaller doses were administered. It is our opinion, however, that the latter is the more efficacious. In addition there is the factor that a tolerance appears to develop to any one foreign protein. As the animal was used for later studies it was noted

that larger and larger amounts of the same substance were necessary to produce an equivalent effect. If, on the other hand, one administered a substance not previously employed such larger amounts were not required. The duration of the insulin resistance also varied from observation to observation. In some instances it was present for only a few days, whereas, in one instance it persisted for 66 days. The duration of the insulin resistance was not related to the amount nor to the method of administration of the foreign protein. The daily administration for several days, however, almost uniformly resulted in the production of insulin resistance. Insulin resistance developed following the subcutaneous injection of turpentine in both instances. It appeared the day of injection,

TABLE 2

DATE	DAILY URINE VOLUME	DAILY URINE DEXTROSE	DAILY POTENTIAL DEXTROSE DIET	DAILY INSULIN UNITS
	<i>cc.</i>	<i>grams</i>	<i>grams</i>	
6/9/39	415	7.0	145	24
6/10	405	6.0	145	24
6/11	340	7.0	145	24
6/12	440	12.0	145	24
6/13	495	11.0	145	24
6/14	480	14.0	145	24
6/15	490	20.0	145	24 100 mil. H. antigen i.v. 100 mil. H. antigen i.m.
6/16	525	21.0	145	24
6/17	470	19.0	145	24
6/18	500	25.0	145	24 200 mil. H. antigen i.v. 200 mil. H. antigen i.m.
6/19	650	37.0	145	24 200 mil. H. antigen i.v. 200 mil. H. antigen i.m.
6/20	775	59.5	145	24 200 mil. H. antigen i.v. 200 mil. H. antigen i.m.
6/21	930	71.5	145	24 200 mil. H. antigen i.v. 200 mil. H. antigen i.m.

Table shows the moderate insulin resistance in dog 6 following administration of 100 million H. antigen intravenously and intramuscularly, and 200 million H. antigen intravenously and intramuscularly daily.

gradually increased until the third day, subsided rapidly for two days and then increased until it reached its maximum two days later at the height of the local reaction. It then decreased as the local irritation subsided. The response to the administration of living *Bacillus coli* culture was very similar to that obtained with typhoid vaccine.

The responses obtained in the two dogs in which small pieces of pancreas were permitted to remain at the original operation were no different from those in which pancreatic tissue was not found at post-mortem at the end of the studies.

One animal was permitted to develop a fatty liver as described by Best and



co-workers (2) and attributed to lipocaic deficiency by Dragstedt (3). Insulin resistance did not develop following administration of larger doses of a foreign protein that had previously produced insulin resistance in this animal. Also

TABLE 3

DATE	DAILY URINE VOLUME	DAILY URINE DEXTROSE	DAILY POTENTIAL DEXTROSE DIET	DAILY INSULIN UNITS
	<i>cc.</i>	<i>grams</i>	<i>grams</i>	
3/6/37	350	3.5	94	10
3/7	420	1.7	94	10
3/8	330	1.0	94	10
3/9	250	1.0	94	10
3/10	300	1.0	94	10
				40 million typhoid i.v. 40 million typhoid i.m.
3/13	440	0.5	94	5
3/17	860	61.0	94	8
3/24	1120	82.0	94	10
3/31	1400	83.0	94	50
4/6	680	31.0	94	80
4/13	925	19.0	94	70
4/20	1200	79.0	94	80
4/27	1430	89.0	94	80
5/4	2000	125.0	119	160
5/11	1260	89.0	94	200
5/16	700	11.0	94	280

Table shows extreme insulin resistance in dog 3 following one administration of typhoid vaccine.

TABLE 4

MATERIAL	NO. OF EXPERIMENTS	INSULIN RESISTANCE			
		None no.	Slight no.	Moderate no.	Extreme no.
Typhoid vaccine.....	9	5	3	0	1
Typhoid H. antigen.....	8	1	1	6	0
Skimmed milk.....	3	1	1	1	0
B. coli culture.....	1	0	0	1	0
Turpentine.....	2	0	0	2	0
Total.....	23	7	5	10	1

Table shows the effectiveness of the different materials to produce insulin resistance.

one dog developed just as much insulin resistance after its liver had been filled with 200 grams of acacia as it did before.

DISCUSSION. These observations demonstrate that insulin resistance can be produced in chronic experiments in depancreatized dogs. They make untenable the theory that there is a decrease of endogenous insulin secretion in such cases. There appears to be a difference in susceptibility to production of insulin re-

sistance in the different dogs. It does not develop in all dogs under comparable conditions. It may occur in one dog with the slightest provocation and may be very difficult to produce in another dog. In this respect the diabetes mellitus in depancreatized dogs appears to be similar to that in man. According to Greene and Keohen (4) insulin resistance does not occur in all cases of diabetes mellitus in man during an infection nor following the injection of typhoid vaccine.

The cause for the insulin resistance is not clear. The possibility that aberrant pancreatic tissue was present in our animals and that the insulin secretion by this tissue was decreased during insulin resistance appears extremely unlikely. There was no difference in the response of two dogs in which pancreatic tissue was permitted to remain and in those in which no pancreatic tissue was found at autopsy. In addition it is doubtful that cessation of insulin secretion by small portions of pancreas could account for the extreme alterations of dextrose utilization observed in some of our experiments.

The theory that the exogenous insulin is less effective during insulin resistance is undoubtedly true, but this does not explain the mechanism of insulin resistance. There are several possible explanations all of which remain unanswered. The animal is either unable to oxidize as much dextrose as previously or to store glycogen in the liver or tissues to the previous extent. A fatty liver is known to prevent the storage of glycogen by the liver, but the presence of a fatty liver did not augment insulin resistance in one of our animals. Likewise filling one animal's liver with an extreme amount of acacia did not increase the amount of insulin resistance producible in that animal.

#### SUMMARY

Insulin resistance has been produced in chronic experiments in depancreatized dogs. The susceptibility to insulin resistance varies from dog to dog and in the same dog from one observation to another. Small pieces of remaining pancreas, the presence of fatty liver and filling the liver with acacia are not factors in insulin resistance.

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# THE RESPIRATION OF THE DEVELOPING BRAIN

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This report deals with the oxygen consumption of different parts of the brains of rats during extrauterine development and the glucose utilization of whole brains at various ages.

**METHODS.** The brain, without the olfactory bulbs, was divided as follows: The pallium and cerebellum were separated from the neuraxis. The latter was cut in two in front of the pons. The caudal part to the first spinal nerve is called the medulla; the rostral part, which includes the corpora striata, is designated as the stem.

Warburg respirometers were used for the measurement of the oxygen consumption. Each of the above parts of the brain was chopped and suspended in tared vessels containing Ringer-glucose solution buffered to pH 7.4 with phosphate (1). The wet weights were determined before placing the vessels in the bath. Care was taken to put approximately the same weight of tissue in all the vessels. Thus with the brains of older rats it was necessary to distribute the pallium and the stem in two or three vessels, while to obtain sufficient tissue in newborn rats it was necessary to pool the parts of the brains of several litter mates. The temperature of the bath was maintained at 38°C. Readings were taken beginning 50 min. after death of the animal. The results are expressed in cubic millimeters of oxygen per 100 mgm. of wet brain per hour.

Glucose utilization was determined on whole chopped brain suspended in Ringer-glucose-phosphate medium (10 cc. of medium for each 200 mgm. of tissue) and shaken in stoppered Erlenmeyer flasks for two hours. Shaffer-Hartman-Somogyi's method was used for glucose determination. The results are expressed as milligram of glucose per 100 mgm. of wet weight per hour.

**RESULTS.** Figure 1a shows the oxygen uptake of the various parts of the brain during growth. In figure 1b is plotted the oxygen uptake of the entire brain during that same period. These latter values were calculated from the oxygen uptake and the weights of the individual parts of the brain. Because of the large relative weight of the pallium its influence on the metabolism of the whole brain is so great that the curve in figure 1b resembles the curve found for the pallium in figure 1a; the influence of the other brain parts is of course in proportion to their relative weights (table 1).

As has been found by others (2), the oxygen uptake of the whole brain is at its lowest level during the first week of life. A slight fall in respiration occurs between the second and fourth day, which is the result of a similar drop that occurs in the pallium and, to a lesser extent, in the stem (fig. 1a). During the

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first two weeks the medulla has the greatest oxygen uptake and the pallium the lowest. No consistent values could be obtained for the cerebellum for the first week because of its minute size, but there are indications that its respiration is of the same magnitude as that found for the medulla. At the end of the first week a sharp rise in the respiration of the pallium and stem occurs, which continues until a maximum is reached at the 7th and 5th week respectively. There occurs during this period a re-arrangement in the order of the rates at which the different parts of the brain take up oxygen. By the fifth week the stem has the highest respiration, followed by the pallium, cerebellum and the

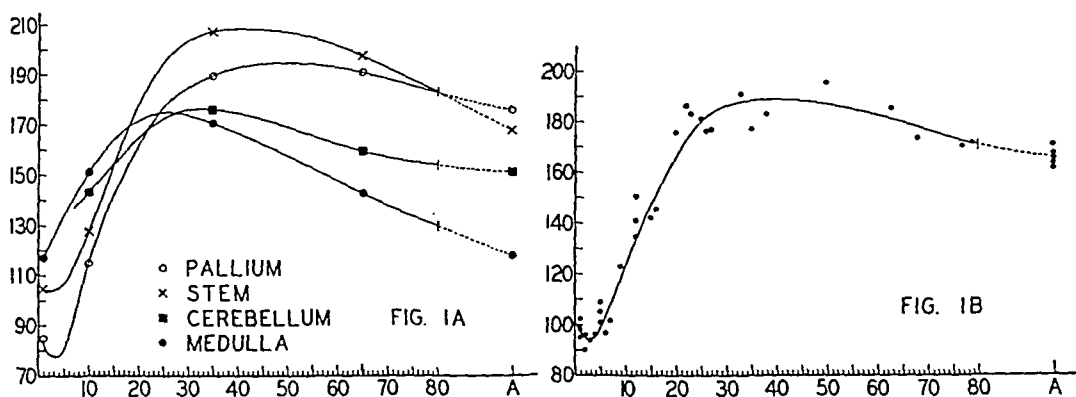


Fig. 1. Oxygen is expressed in cubic millimeters per 100 mgm. of wet brain tissue per hour. Time is given in days. A stands for adult.

In figure 1a the points are identification points for the curves of the various parts; they do not represent points of observation. In figure 1b each point is an observation.

TABLE 1  
*Weight of various parts of the brain (whole brain is 100)*

AGE	PALLIUM	STEM	MEDULLA	CEREBELLUM
1-7 days	58	25.5	12.5	4
21-28 days	61	17	11	11
Adult	55	19	14	12

medulla. The medulla showing a steady rise from birth reaches a maximum at about the third week, which is earlier than that of stem or pallium.

The greatest oxygen uptake of the whole brain is found between the 4th and 7th week and then falls off gradually until at about the 20th week the adult level is reached. The respiration of the stem drops below that of the pallium at about the 11th week, so that when the adult level is reached the brain parts take up oxygen in the following order: pallium stem, cerebellum and medulla. This confirms results on adult rats by Himwich, Sykowski and Fazekas (3).

The glucose utilization of three age groups is shown in table 2. It is lowest in the 1 to 10 day old rats, highest in the 10 to 30 day old groups and falls off slightly for the adults. Expressing the glucose and oxygen data in micro-

equivalents (columns IV and V) we find, however, that the ratio of glucose disappearing over the oxygen utilized is greatest in the 1 to 10 day old group (column VI). This glucose oxygen ratio is undoubtedly an index of the amount of glycolysis.

**DISCUSSION.** The curves of the oxygen consumption of the various parts of the developing rat brain resemble curves reported for dogs (4). In rats there can be distinguished three levels of metabolism during the postnatal life. An examination of the results of Himwich and Fazekas reveals that the same three levels can be found also in dogs. The metabolism during the first week of life is characterized by a very low oxygen and glucose utilization and a high glycolytic activity (first level). Between the 4th and the 7th week the respiration is the highest to be found during the animal's life (second level). The third or adult level is lower than the second one and is reached after about 20 weeks. The individual parts of the brain exhibit also these three levels although all parts do not reach these levels at the same time. Thus the curve of the medulla indicates that at birth the respiration of this part already is approaching the second level,

TABLE 2  
*Utilization of glucose by the whole brain*

AGE	GLUCOSE UTILIZATION IN MGM./100 MGM. BRAIN TISSUE	O <sub>2</sub> UTILIZATION IN mm. <sup>3</sup> /100 MGM. BRAIN TISSUE	GLUCOSE UTILIZATION IN MICRO EQUIVALENTS/100 MGM. BRAIN TISSUE	O <sub>2</sub> UTILIZATION IN MICRO EQUIVALENTS/100 MGM. BRAIN TISSUE	RATIO OF GLUCOSE O <sub>2</sub> UTILIZATION
1-10 days	0.66	100	22	4.5	5.1
10-30 days	0.94	180	31	8	3.9
Adult	0.86	165	28.5	7.5	3.8

while the pallium and the stem remain at the first level for several days after birth.

The slight drop in metabolism of the pallium and the stem between the 1st and 4th day is probably due to the hydration that occurs at this time. Donaldson and Hatai (5) reported that the amount of dry material of the brain falls from 12.6 per cent to 11.9 per cent during the first 4 days of life. This indicates that during that period about 60 mgm. of water have been added to each gram of brain tissue. The addition of such considerable amounts of non-respiring material may account for the drop in oxygen uptake. Although this decrease in solid material also occurs in the medulla, the rise in metabolism in that part no doubt masks the drop to be expected from hydration. Similarly the fact that the drop in the pallium is greater than that of the stem may indicate that the rise in the oxygen uptake begins earlier in the stem than in the pallium.

It is interesting to note that the metabolisms of the various parts reach their peaks in the same order as the transition of the first into the second level takes place, namely, first the medulla (end of the third week), then the stem (end of the fifth week) and finally the pallium (seventh week).

The rise in metabolism coincides with the beginning of myelinization of the

rat's brain (6, 7, 8). This process continues with great intensity until about the 6th week of life (7). It is remarkable that notwithstanding the deposition of considerable amounts of poorly respiring myelin (9) the respiration of the rat brain almost doubles during that time. It might be assumed that the energy required for myelinization and the many other changes occurring during the first month of life (7) results in the high metabolism found during this period. However, the fact that the oxygen consumption does not fall off to the same extent as the reduction of the formation of myelin and other substances, but remains, at least in the stem and pallium, at a continuous high level forestalls this conclusion. The order in which the brain parts start their transition from the first into the second level, and in which they reach their peaks, is the same as the order in which they start functioning. It seems likely, therefore, that the transition is correlated with the beginning of function of the brain parts.

The high glycolytic activity found during the first week of life is of considerable interest in view of the high resistance that newborn animals show to oxygen lack and circulatory arrest (10). It has been suggested (11) that newborn animals can obtain anaerobic energy through glycolysis to a greater extent than the adults.

#### SUMMARY

1. The oxygen consumption and glucose utilization of excised rat brain were determined at different ages. Three distinct levels in the respiration of the brain can be distinguished. The lowest level which occurs during the first week of life is characterized by a low oxygen and glucose utilization but a high glycolytic activity. The highest level occurs between the 4th and 7th week and is characterized by a high glucose and oxygen utilization but a decrease in glycolytic activity. The adult level is reached at about the 20th week and is somewhat lower than the second level.

2. The order in the rate at which the various parts of the brain take up oxygen changes during development. At birth the medulla has the highest respiration, followed by stem and pallium; in the adult, this order is reversed.

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## AN ACID-NEUROHUMORAL MECHANISM OF NERVE CELL ACTIVATION<sup>1</sup>

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The observations reported in this paper present an unlooked for variation of the phenomenon of after-discharge which promises broad application in the field of neurophysiology. This modification was fortuitously revealed in the after-hyperpnea produced by faradic stimulation of Hering's nerve and of the cutaneo-sensory saphenous nerve of the dog. (Morphine urethane anesthesia and both cervical vagi cut.)

Originally the purpose of this experiment was to compare the phenomenon of after-discharge in a respiratory reflex arc with that of the spinal reflex arcs, so well established by Sherrington and his co-workers (1906, 1932). It appeared to us that a demonstrable basic similarity of these two forms of motor integration would permit a more intimate application of the great store of knowledge on spinal integration and thus enhance our understanding of the respiratory act.

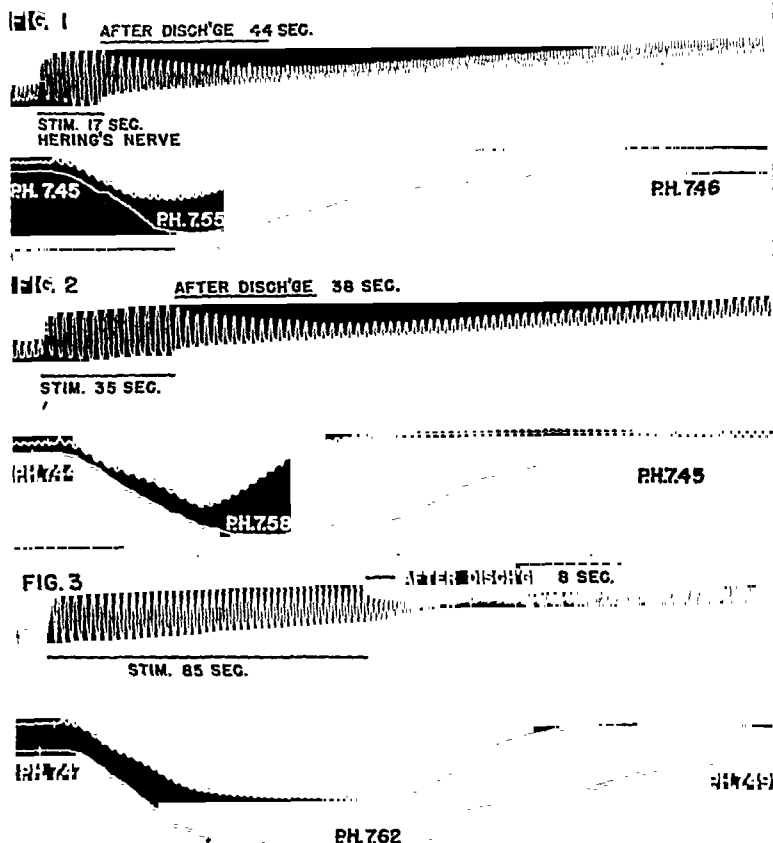
It was to be expected that the reflexogenic hyperpnea elicited by faradic stimulation of chemoceptive and sensory cutaneous afferents would overventilate the blood and tissues and produce a general drop in the hydrogen ion concentration. A continuous record of the changes in cH of the circulating blood (glass electrode in the path of the carotid flow, see Brassfield, 1936) was, therefore, indicated to show the extent and progress of these changes; for a reduction of cH or of CO<sub>2</sub> pressure might, in accordance with the accepted stimulating action of increased cH, be expected to oppose the after-action of reflexogenic stimulation and abbreviate the after-discharge. Therefore, if hyperpnea were found to continue after artificial stimulation had ceased, i.e., during a reflexogenically established hypocapnia and hypo-acidity, it would show in a most decisive way the tendency of nerve cells to continue to discharge in the absence of the original impinging excitatory impulses. This tendency is clearly revealed for the respiratory reflex arc in figure 1. Though the arterial pH rose from 7.44 to 7.55, hyperpnea nevertheless continued for approximately 45 seconds after stimulation of Hering's nerve had ended.

Our main purpose—that of showing a marked similarity of after-discharge in the respiratory and spinal reflexes (see Gesell, 1940)—having been served the problem was for the time being dropped. Only later, on comparing observations on the effects of varying periods of stimulation was it indicated that a more significant principle had been uncovered (see figs. 1, 2 and 3). In figure 1, where stimulation lasted only 17 seconds the phenomenon of after-hyperpnea persisted for a period of 44 seconds. When stimulation was increased to 35 seconds the after-discharge shortened to 38 seconds. And finally when stimula-

<sup>1</sup> Preliminary Report—Univ. Hosp. Bull. (Michigan) 7: 94, 1941.

tion was markedly prolonged to 85 seconds the after-hyperpnea lasted only the short period of 8 seconds.

Such striking inverse relation of after-discharge to stimulation stands in marked contrast to the firmly established findings of Sherrington (1906, 1932) in which spinal reflex after-discharge varies directly with the amount of stimulation. The absence of hyperventilation in the experiments of Sherrington seems to be the critical difference involved and very pointedly indicates a most important rôle of cH in neurophysiology. How then does acidity modify the



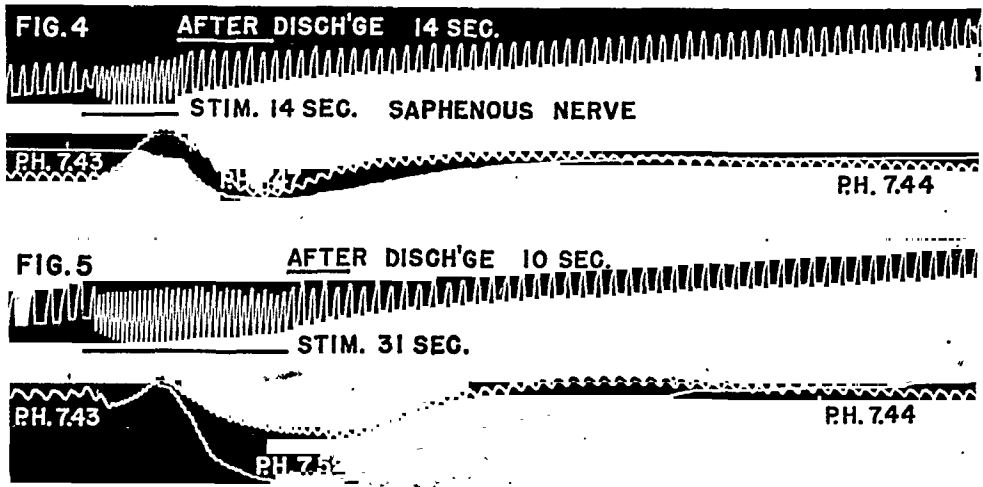
Figs. 1, 2 and 3. An inverse relation of the duration of after-discharge to the duration of faradic stimulation of Hering's nerve. Sherrington's criterion of the return of contraction to the original intensity is employed to determine the duration of the after-discharge.

activity of nerve cells? One possible explanation is that already advanced by Gesell, Lapidès and Levin (1940) for the respiratory center, according to which nerve cell activity is a resultant of two forms of stimulation: 1, the direct action of increasing cH upon the nerve cell itself, plus 2, the stimulating action of nerve impulses. Hyperventilation would thus theoretically subtract a fraction of the direct stimulating action of cH from the sum-total of 1 + 2. Gesell, Lapidès and Levin (1940) offer a simple electrotonic mechanism by which the addition and subtraction of 1 and 2 may be accomplished.



Without suggesting the elimination of the direct stimulating action of cH on nerve cells as an integrating force we see potent reasons for consideration of an additional mechanism in which increasing cH exerts an indirect stimulating control. On the assumption that nerve cells within the brain are reflexogenically excited by a physiological deposition of acetylcholine (Dale, 1935), as is now recognized for the outlying ganglia, the instability of acetylcholine in alkaline solution repeatedly observed by Dale and his co-workers calls for our attention. In figure 1 where the stimulation of Hering's nerve is shortest and the increase of pH the smallest, the after-discharge is longest. In figure 2 where the increase in pH is somewhat greater the duration of the after-discharge is shorter. In figure 3 where the pH rises to the highest level the after-discharge is shortest.

Granting that the instability of physiologically deposited acetylcholine increases in an increasingly alkaline physiological media we are faced with a new



Figs. 4 and 5. An inverse relation of the duration of after-discharge to the duration of faradic stimulation of the saphenous nerve.

and simple mechanism of an acid-neurohumoral control of nerve cell activity and incidentally of the control of respiration. *By controlling the life span of a single deposit of acetylcholine, acidity thus determines the value (the duration and intensity of action) of that corresponding impulse. By virtue of the same principle acidity would determine the sum-total of existent acetylcholine deposited by the myriads of impinging signals, and thereby the sum-total of stimulation at any given moment and the duration of after-discharge following cessation of inflowing impulses.* That in short is our theory of the acid-neurohumoral mechanism of gradation of nerve cell activity.

Variations in duration of stimulations of the saphenous nerve though eliciting a hyperpnea in which accelerated rhythm predominated produced the same type of changes in the after-discharge described for Hering's nerve (see figs. 4 and 5). Stimulation of only 14 seconds in figure 4 increased the pH from 7.43

to 7.47 and produced an after-discharge which lasted approximately 14 seconds whereas in figure 5 where stimulation continued for 31 seconds and raised the pH from 7.43 to 7.52 the after-discharge endured for only 10 seconds.

Such striking similarity of results obtained on nerves of such diversity of specialization and function is of interest in suggesting that the acid-neurohumoral mechanism is not confined to afferent impulses solely related to the respiratory act in which the importance of cH as a regulatory factor has long been recognized. In fact recent experiments indicate the existence of an acid-neurohumoral mechanism at all central and peripheral junctions wherever acetylcholine is physiologically deposited (Gesell, Brassfield and Hansen, 1941; Hansen, Worzniak and Gesell, 1942; Brassfield and Gesell, 1942; Mason and Gesell, 1942; Gesell, Brassfield and Hansen, 1942). Thus we have discovered in a purely accidental way a mechanism which Winder (1938) and Worzniak and Gesell (1938) had sought by a direct approach. Winder had attempted to show the interaction of acidity and acetylcholine in the activity of the carotid body but failing to obtain conclusive evidence tentatively dropped the experiments on the chemoreceptors. Worzniak and Gesell studied the possibilities of an acid-neurohumoral mechanism of nerve cell activation in the respiratory center. Although several procedures gave indication of a complementary action between acid and acetylcholine their results were also regarded as suggestive rather than conclusive. Only in retrospect do these findings appear more substantial than was realized.

It is strange that a mechanism which, in the light of increasing evidence, seems so obvious should have eluded recognition so long. The instability of acetylcholine in alkaline solution was well-known. So was its destruction by cholinesterase. Furthermore, Plattner, Galehr and Koderä (1928) showed that the rate of destruction of acetylcholine by cholinesterase in blood serum or in a suspension of red blood cells increased with increasing alkalinity in the range of pH 5.6 to pH 8.2. Feldberg and Schriever (1936) found that asphyxia led to the appearance of acetylcholine in the cerebrospinal fluid of a lightly eserized animal but they make no mention of the undoubted increase of cH of the brain and the preservative action which this might have had. They conclude their paper as follows "We have as yet no data which would warrant speculation as to the relation between this output of acetylcholine after adrenaline and asphyxia and any functional changes in the nerve centers". Andrus (1924) finds that the inhibitory action of acetylcholine on the rabbit's auricle is greater at a pH of 7.0 than at a pH of 8. Stimulation of the vagus nerve was also more effective at a pH 7.0. Clark (1927) describes a decreasing effectiveness of acetylcholine on the ventricle of the frog with increasing alkalinity.

The slowness of destruction of acetylcholine, generally regarded as the strongest argument against the neuro-humoral theory (see Eccles, 1936) must now be reconsidered in a new light for it seems highly probable that fluctuations in the rate of disappearance of acetylcholine is part and parcel of an elastic mechanism of gradation of nerve cell activity.

## SUMMARY

The duration of respiratory after-discharges following faradic stimulations of either Hering's nerve or the cutaneo-sensory saphenous nerve varied inversely with the duration of stimulation. Such findings are contrary to the direct relation of after-discharge to stimulation as found by Sherrington in spinal reflexes. These differences in respiratory and spinal after-discharges were attributed to the alkalinizing action of the reflexogenic hyperpnea. Since acetylcholine in alkaline media and in the presence of cholinesterase is very unstable it has been proposed that variations in cH may determine the duration of the life span of each deposit of acetylcholine produced by each impulse impinging on a nerve cell. Variations in the life span of individual deposits would in turn determine the rate and amount of accumulation of acetylcholine at nerve cells under bombardment, as well as the rate at which accumulated acetylcholine disappeared when bombardment ceased. Prolonged hyperpneas which turned the tissues more alkaline than shorter hyperpneas might, therefore, be followed by shorter periods of after-hyperpnea. Findings conform with theory. It has been suggested that the control of the longevity of acetylcholine deposits by variations in cH may prove to have an important rôle in neurophysiology.

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# THE SPONTANEOUS ACTIVITY AND FOOD INTAKE OF RATS WITH HYPOTHALAMIC LESIONS<sup>1</sup>

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Bailey and Bremer (1921) speak of "apathy" in their two obese dogs which had hypothalamic lesions, and several of Brown's (1923) fat dogs became "sluggish." Smith (1930), evidently referring to certain early postoperative symptoms, stated that rats which had received an injection of chromic acid into the hypophysis, presumably injuring the hypothalamus, like other rats with hypothalamic lesions, displayed periods of quiet interspersed with periods of excitement. Speaking also of rather early postoperative activity, Krieg (1938) mentioned, among other manifestations, an "emotionally hyperactive state," and "depression."

Although these and similar incidental references have been made by various workers to the behavior and activity of animals with damage to the tuber cinereum, apparently with but one exception (Richter, 1930) no one had tried until very recently to compare quantitatively the gross activity spontaneously exhibited by such animals with preoperative or normal values. Richter produced diabetes insipidus in seven rats by stabbing a short narrow blade through the sphenoid bone at a point he judged to be just rostral to the hypophysis. As yet the nature of these hypothalamic lesions, if indeed such they were, has not been described; but they probably occupied an area in the neighborhood of the median eminence. The result of this procedure was found to be essentially negative. Rats which had run well before the operation continued to do so afterward, and the poor runners remained inactive.

Food intake of these animals, Richter found, was likewise unchanged. Of course, none of the rats observed in these experiments became obese and it may be assumed that they did not bear the sort of hypothalamic lesions attributed by Hetherington and Ranson (1940) with the capacity to cause adiposity.

Other investigators, however, have reported a contrary finding. Keller, Hare and D'Amour (1933) and Keller and Noble (1935) observed enhanced food intake and a tendency to adiposity both in dogs with hypothalamic lesions and in dogs with a variety of pituitary injuries. Ranson, Fisher and Ingram (1938) noticed that one of their monkeys with hypothalamic lesions increased rapidly in weight and displayed marked polyphagia.

Finally in two simultaneous preliminary reports Tepperman, Brobeck and Long (1941) and Hetherington (1941) have added the most recent information on the subject. The former group found that certain rats with hypothalamic lesions became extremely obese and consumed approximately twice as much food

<sup>1</sup> Aided by a grant from the Committee on Research in Endocrinology of the National Research Council.

as litter-mate controls. Hetherington, on the other hand, observed a group of operated rats which did not eat more than their controls, but which, nevertheless, in some instances did grow fat. The latter's animals were observed, in addition, to engage in a great deal less spontaneous running activity than did the controls.

The following experiments contain an elaboration of the results reported by Hetherington (1941) and a considerable amount of new material which has been added since that time.

**METHODS.** The present series numbers 18 male rats, 11 operated animals and 7 controls, run in groups of 6. Each group was made up of litter-mates. One control and one operated animal failed to survive the full length of time covered by the experiments and are not included in the results. Hypothalamic lesions were placed in the operated rats by the method described by Hetherington and Ranson (1940).<sup>2</sup>

For study of spontaneous running activity the type of cage, with slight modifications, described by Richter and Wang (1926) was used. The modifications were as follows: The living compartment is considerably smaller on these cages, being 3 inches wide by 5 inches high by 6 inches long. The revolving drum is a little larger, having a diameter of 15 inches. It is balanced in order to enable it to be stopped at any position, and revolves so easily that a weight of less than half a gram at the periphery will cause it to turn.

The cages were not kept in an air-conditioned room; consequently humidity undoubtedly varied over a considerable range. The temperature of the room, however, was kept within the range between 75° to 78°F. (Animals with hypothalamic lesions generally require a warmer room than normal for maintained good health.) They were subjected to 12 hours of illumination and 12 hours of darkness per day, the lights being controlled by a Tork electric clock.

The food supply was altered from group to group in the following manner: The first group received nothing but Rockland Rat Ration pellets for the first 5 weeks of the experiment. During the final 3 weeks the pellets were ground and moistened with water. The other two groups received a mixture made of 37 per cent ground Rockland rat pellets, 18 per cent ground dry white bread, and 45 per cent raw whole milk by weight. Needless to say, samples of this diet (as well as of the pellets first used) were dried daily to determine their moisture content; and all figures given in the results are dry weights calculated from the dried food residue collected at the end of each 24-hour period.

To determine the influence of activity upon food intake the second two groups were kept part of the time in small cubical living cages (7½ in. on a side), and the remainder of the time in the activity cages. One group spent the first 6 post-operative weeks in the activity cages and the next 4 or 5 weeks in the stationary cages. The process was reversed with the other group, the first 5 weeks being spent in the ordinary living cages and the final 4 weeks in the activity cages.

**RESULTS.** The lesions found in the hypothalami of the operated rats need not be described in detail at this time. It is sufficient to say that they conformed

<sup>2</sup> For the Evipal anesthetic used in the operations we are indebted to Dr. J. J. Kuhn of the Winthrop Chemical Co.

in a general way with the lesions found in a former series (Hetherington and Ranson, 1940). As before, lesions producing the higher degrees of adiposity in this series lay on both sides in the region of the ventromedial hypothalamic nucleus and its immediate cellular environs. Lesions failing to precipitate the syndrome, or causing it to only a minor degree were rather markedly asymmetrical, not near enough to the base, or for some other reason inadequate.

When the experiment with each group of animals was terminated, the rats were weighed, anesthetized, and their body length (nose-anus) measured. They

TABLE 1

*Table summarizing the data on age, weight, and body length of the rats at the time of the termination of the experiment*

RAT NO.	OP. OR CON.	AGE	WEIGHT	NOSE-ANUS LENGTH	$W^{\frac{1}{3}}/L$	DEGREE OF ADIPOSITY
		days	grams	cm.		
Rf-1	C	133	385	24.7	0.294	
Rf-2	O	133	380	23.8	0.304	+
Rf-3	C	136	373	24.3	0.296	
Rf-4	O	133	342	23.1	0.303	?*
Rf-5	O	136	347	20.5	0.342	+++
Rf-6	O	136	325	23.1	0.297	-
Rf-7	O	172	485	23.8	0.330	+++
Rf-8	C	172	413	25.1	0.297	
Rf-9	C	157	371	23.9	0.300	
Rf-10	O	157	473	24.3	0.321	+++
Rf-11	O	157	449	23.4	0.327	++
Rf-12	C	died	.			
Rf-13	O	153	413	22.9	0.325	+++
Rf-14	O	died				
Rf-15	C	153	323	23.1	0.297	
Rf-16	C	153	335	23.0	0.302	
Rf-17	O	153	349	23.5	0.299	-
Rf-18	O	153	331	22.5	0.307	?

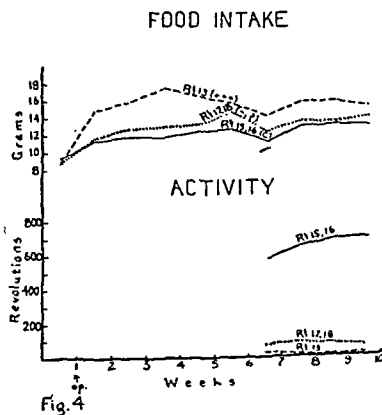
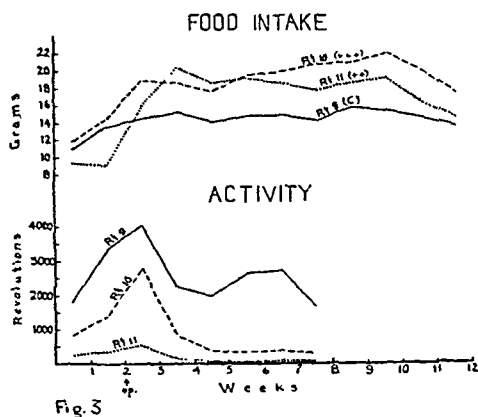
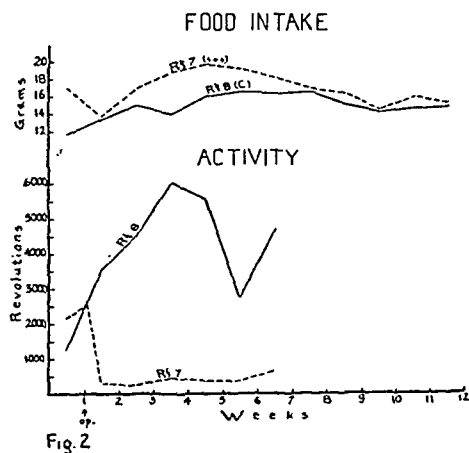
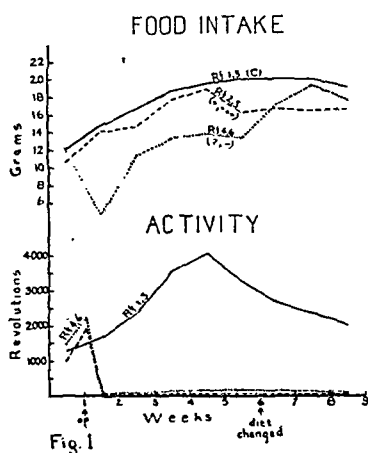
\* Indicates presence of adiposity doubted.

were not always immediately killed. The data obtained at this time are summarized in the table, which shows age, weight, and body length, and several indices of degree of adiposity which will now be explained.

The formula  $W^{\frac{1}{3}}/L$ , expressing the ratio of the cube root of the body weight in grams to the body length in centimeters, was borrowed from Lee (1929), who was interested in the expression of metabolic results for white rats. Following Cowgill and Drabkin (1927), who applied the formula to the dog, Lee used this "nutritive correction factor" to indicate the nutritive state observed in an individual animal.

In the table opposite the weight-length ratio of each operated rat is placed a

symbol, either a minus-sign, or one, two, or three plus-signs, which represents a visual estimate of the degree of an animal's adiposity. This estimate was based on a careful inspection and comparison of the rat with its control, and signifies that the animal was judged either not to be fat, or to be slightly, moderately, or markedly obese. The two classifications of adiposity have not always given results completely consistent with each other, yet they do not in any case fundamentally disagree.



Figs. 1-4. Records of food intake and spontaneous activity (running) of obese and nonobese rats with hypothalamic lesions, and of their normal litter-mate controls. In figure 1 the change in diet at the end of the fifth postoperative week should be noticed. In figure 4 note the vertical scale for activity is different from that in the first 3 figures.

No attempt has been made to divide the weight-length ratios arbitrarily into groups. Inspection of the table will reveal, however, that no control rat exhibits a ratio above 0.302; the average of the ratios of the 6 normal males is 0.298. Determined for a much longer series of normal males used in other experiments the figure is slightly lower—about 0.293—with an upper limit to the range of normal values, as here, at about 0.302. No male which was considered definitely obese has a ratio below 0.304, and the fatter animals have ratios above 0.320.

All data secured from the activity cage experiments and from determinations of food intake are summarized graphically in figures 1 to 4.

The first 6 animals were placed in activity cages at the age of 6 weeks, and 4 of them were operated 2 weeks later. Two of the operated rats in a matter of 4 to 6 weeks showed an unmistakable degree of adiposity, while the other 2 retained an essentially normal appearance. In figure 1 the mean daily food consumption of each rat for any given week is averaged together with the mean daily food consumption of the other fat rat for the same week, and the average of the means is plotted on the graph as a single point. The same procedure is followed with the figures for daily activity, and is applied to the corresponding determinations for the pair of controls and the pair of nonobese operated animals. This has been done purely to reduce for the sake of simplicity and clarity the number of lines on the graph. With particular reference to the upper set of lines in figure 1 the point should be stressed at this time that the paired representation of food intake does not conceal an overlapping of the data of the normal and obese rats. Neither of the fat animals ate as large an amount of food as either of their control litter-mates.

As was mentioned before, the diet of these animals consisted during the first 5 postoperative weeks of whole Rockland rat pellets. On this diet the 2 nonobese rats maintained a considerably lower level of food intake than the others until the final 3 weeks of the experiment, when grinding and moistening of the food pellets (with water) seemed to exert a favorable influence upon their food consumption. The change apparently did not induce the 2 obese and 2 normal animals to alter their eating habits.

With regard to spontaneous running activity of the animals, the graph (fig. 1) speaks for itself. In this set of animals, and indeed in all the others as well (figs. 2-4), the trend is clear. Rats with large hypothalamic lesions in the region dealt with here evidently indulge in a great deal less running activity than do the majority of normal animals, or than they themselves did previous to the placing of the lesions. The change is striking and practically immediate, occurring within one or two days after the operation. There is a suggestion that the obese animals are even more inactive than those which do not grow fat (figs. 1, 4), but in view of the small number of animals tested, the difference might not be significant.

Figures 1 to 3 illustrate a state of hyperactivity which usually appears during the acute postoperative stage. Often rats with large lesions in this region of the hypothalamus will run almost continuously in an automatic, almost frenzied fashion for several hours after they awake from the anesthetic. This period may be succeeded by another lasting several days when the animals will seem to be stuporous, but will respond with exaggerated violence to slight tactile stimuli. The phenomenon was noticed many times in this laboratory, even long before the work on activity was begun. After the acute phases of the postoperative period are past the rats are rather lethargic, though this characteristic usually does not appear to partake of somnolence or a lack of alertness. They are, in fact, generally somewhat irritable and excitable for a number of weeks. With handling the rats after a time often lose this touchiness to a certain extent. (It should be mentioned, in passing, that more recently operated rats with lesions in the caudal hypothalamus have displayed neither the acute hyperactivity nor



the later hyperirritability of the rats just described. As a matter of fact, they tend to be rather amiable and more passive than normals. Even rats with the more rostrally located injuries will display the symptoms to a much slighter degree if the lesions in question are small.)

In a preceding section it was explained that the second and third groups of animals were fed a mixture of ground Rockland rat pellets, ground dry white bread, and raw whole milk. Food intake on this diet may now be considered.

Figure 2 shows how one operated rat which was accustomed to eating somewhat more than its control even before placing of the lesions, maintained, or even widened the margin between its food intake and that of its control after operation. After the animals were removed from the activity cages the food intake of the pair became more nearly equal.

In figures 3 and 4 is to be found a much clearer demonstration of the fact that these obese rats with hypothalamic lesions under certain circumstances will consume a good deal more food than do their litter-mate controls.

The final experiment, consisting of a comparison of the food intake of the animals during their sojourn in the activity cages, where it seems likely more exercise is taken by normal rats, with the food intake observed during the period spent in ordinary cages, has been rather inconclusive. In figure 2 there appears to be a slight decline in the food intake of both the normal and the obese rats after removal of the animals from activity cages. There furthermore seems to be a smaller difference between the intakes of the 2 rats, for which the more rapid descent of the curve for the fat animal is responsible. In figure 4 (where again the method of averaged daily means for the pair of controls and the pair of non-obese operated animals is used) is shown a small increase in food intake which occurred when the animals were placed in activity cages.

The rats represented in figure 3, however, reacted at first—apparently somewhat illogically—with a slightly increased food consumption after being transferred from their activity cages. Later these animals, too, decreased intake, and again the margin between the obese and the control rats narrowed because of the more rapid decline in intake by the fat rats. In any case, the changes in food intake which may be associated with the changes in spontaneous activity assumed here are not at all of the same order of magnitude as those which occurred in some of the obese rats, or even in the normal controls during the phase of rapid growth.

**DISCUSSION.** The phenomenon of adiposity is often regarded as a problem involving as causes either lack of exercise or over-eating, or a combination of the two. Doubtless in many cases these simple and easily understood factors may be an adequate explanation for excessive weight. In many other cases, however, as for instance in hypothyroid obesity, uncontrollable adiposity following pregnancy, and Cushing's syndrome, some more fundamental cause, intimately tied in with pathological physiology must be sought. (For critical analyses of recent theories regarding "exogenous" and "endogenous" obesity, see reviews by Wilder, 1938, and Bauer, 1941.)

Similarly, in the case of the experimental hypothalamic obesity being investi-

gated here, the most obvious explanations, decreased activity and augmented food consumption, were first taken up for examination, with the results that have been cited. It is true that these animals exercise a great deal less than normal rats. Yet animals which have somewhat similar lesions but which do not grow obese also indulge in much less spontaneous activity, though perhaps not as little as fat rats. It is also true that under certain circumstances, as when the food is softer, easier to eat, and possibly more palatable, the obese animals will consume excessively large amounts of it. This observation does not alter the fact, however, that these rats will also grow fat—though in all likelihood more slowly—even when food intake is limited to an amount equal to or even a little smaller than that of normal litter-mates.

When the simultaneous reports of preliminary work by Tepperman, Brobeck and Long (1941) and Hetherington (1941) appeared, the former group (who had done little or no work on activity) emphasized the high food intake of their animals; whereas the latter was more impressed by the tremendously decreased activity of the obese rats. Insistence upon the primary importance of either viewpoint would in all probability represent over-simplification of the problem, and this for at least two reasons.

In the first place, the two factors are complementary in their effect upon body weight. Both would tend to increase it. A very sedentary life, combined with a high caloric intake would seem to be an ideal combination for building up a thick panniculus adiposus.

Secondly, these two factors may be only symptomatic, and not fundamental. It is not difficult to imagine, for example, a condition of hidden cellular semi-starvation caused by a lack of easily utilizable energy-producing material, which would soon tend to force the body either to increase its general food intake, or to cut down its energy expenditure, or both. In this connection, it would be of great interest to determine whether these animals exhibit a preference for any particular class (chemically speaking) of foods.

It should be clearly realized that the apparent reluctance of the bodies of these fat rats to utilize their tremendous stores of fat is only relative and not absolute. Brobeck (1941) has stated that his fat rats can be fasted down to a normal body weight; and it has been noticed many times in this laboratory that a rat can stop eating and lose up to a third or more of its body weight before it recovers its appetite. These animals can, therefore, use fat as a source of energy if necessary, though perhaps at a low level of efficiency. The concept which comes to mind is one much like that expressed by Thomson (1938), who, discussing a somewhat similar matter, speculated upon the varying availability to the cell of different substrates of energy-furnishing material.

Evidence for a more basic disorganization of the physiological economy of these animals is not voluminous, but it is suggestive. To begin with, Hetherington and Weil (1940) showed that there was a pronounced deficit in the total body phosphorus of the obese rats with hypothalamic lesions. Although there was a co-existing calcium deficiency as well, the calcium-phosphorus ratio was irregularly altered in such a way that it was believed other phosphorus containing

materials besides bone had suffered. The importance of phosphorus in fat metabolism, particularly fat transport, and as a constituent of numerous physiologically important organic compounds in the body hardly requires elaboration.

In addition, Tepperman, Brobeck and Long found that the basal oxygen consumption of obese rats which had been either fasted for a long time, or pair-fed with their controls was low compared to the normals; while R.Q. determinations in the absorptive state were higher. Daily creatinine excretion was high in their rats, and carbohydrate metabolism in some instances appeared to be affected. Grafe and Grünthal (1929) found lowered B.M.R.'s in obese dogs which were supposed to have hypothalamic lesions.

It is argument along these lines which has suggested the imperative need for further fundamental physiological and biochemical research on rats displaying hypothalamic obesity. The ease with which the syndrome can be produced in the rat, and the wide variety of physiological techniques which can readily be applied to the animal make it an ideal subject for investigation.

#### SUMMARY

The spontaneous running activity both before and after operation of 10 rats with hypothalamic lesions frequently causing adiposity and of 6 normal litter-mate controls has been investigated. In addition the food intake of all the animals has been measured, in some cases both in activity cages and in ordinary cages.

It has been found that animals having bilateral lesions in the medial hypothalamus in the region in and around the ventromedial hypothalamic nucleus tend to indulge in much less spontaneous running than do the majority of normal controls, or than was exhibited preoperatively.

Food consumption of the obese operated animals may greatly exceed the intake of the normal litter-mate controls or may not exceed it at all, depending upon the nature of the food supplied. A soft palatable diet encourages maximum consumption, and a hard dry pellet diet apparently discourages high intake. Animals probably grow obese more rapidly on the former type of diet. The idea is suggested that the obese animal's efforts to increase food intake and cut down energy expenditure are indicative of a partial inability on the part of its physiological mechanism to metabolize easily all of its available food stores.

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# MUSCLE TREMORS AND THE DEVELOPMENT OF TEMPERATURE REGULATION IN BIRDS

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The term muscle tremors, as here used, refers to the coördinated, semi-rhythmic partial muscle contractions or changes in muscle tone which, when pronounced, produce visible shivering. It is generally recognized that tremors are important in temperature regulation of homoiothermic animals. However, it is still a matter of debate as to whether variations in muscle activity alone are sufficient to account for increased metabolism accompanying a decrease in environmental temperature, or whether extra-muscular (i.e., "chemical regulatory") mechanisms are also involved (1). In connection with recent studies (2) on the heart rate of small birds utilizing a specially designed apparatus, the cardio-vibrometer, data were obtained which indicated a close correlation between ontogenetic development of temperature control and the development of definite, coördinated muscle tremors.

**METHODS.** A description of the cardio-vibrometer<sup>1</sup> has been recently published (2, 3). In the experiments with young birds, nestlings of known age were removed from their natural nest and placed in a simulated nest attached to the sensitive piezo-electric crystal (the pickup device); in this way vibrations whether due to heart beat, breathing, or skeletal muscle activity could be recorded (with crystal driven pen writing on moving paper) with a minimum of disturbance to the bird. The amplifier and recorder were designed to record low frequency vibrations up to 60 per second. From the graphic records thus obtained tremors could usually be distinguished from heart beat, breathing, or other recorded movements; furthermore, tremors could be detected in this way when intensity was not great enough to produce visible shivering. All records were made under standard ("basal") conditions in which birds were maintained in darkness, 2 to 4 hours after last feeding, and at a controlled temperature. Usually the pickup and the bird in the sound-proofed temperature chamber were placed in a different room from that containing the recorder and observer. Nestlings of the house wren (*Troglodytes aedon*) and the black-capped chickadee (*Penthestes atricapillus*) were largely used, although a few experiments were run on precocial species including the ring-necked pheasant (*Phasianus colchicus*), common tern (*Sterna hirundo*) and the domestic fowl.

**RESULTS.** Figure 1 is a reproduction of actual cardio-vibrometer records (vibrograms) of two ages of nestling house wrens when resting quietly at standard conditions. The house wren, like other altricial birds, recapitulates during the short period of its nest life the evolutionary development from the poikilother-

<sup>1</sup> The construction of a new and improved apparatus has been made possible by recent grants from the A.A.A.S. and the University Research Center of Georgia.

mic physiology of reptiles to the homoiothermic physiology of birds, the most rapid changes taking place between 4 and 12 days after hatching (Kendeigh, 4). In figure 1, A, B, C are typical records of newly hatched (0-day) nestlings at progressively decreasing temperatures (95°, 80°, 70°F. or 35°, 26.7°, 21.1°C.).

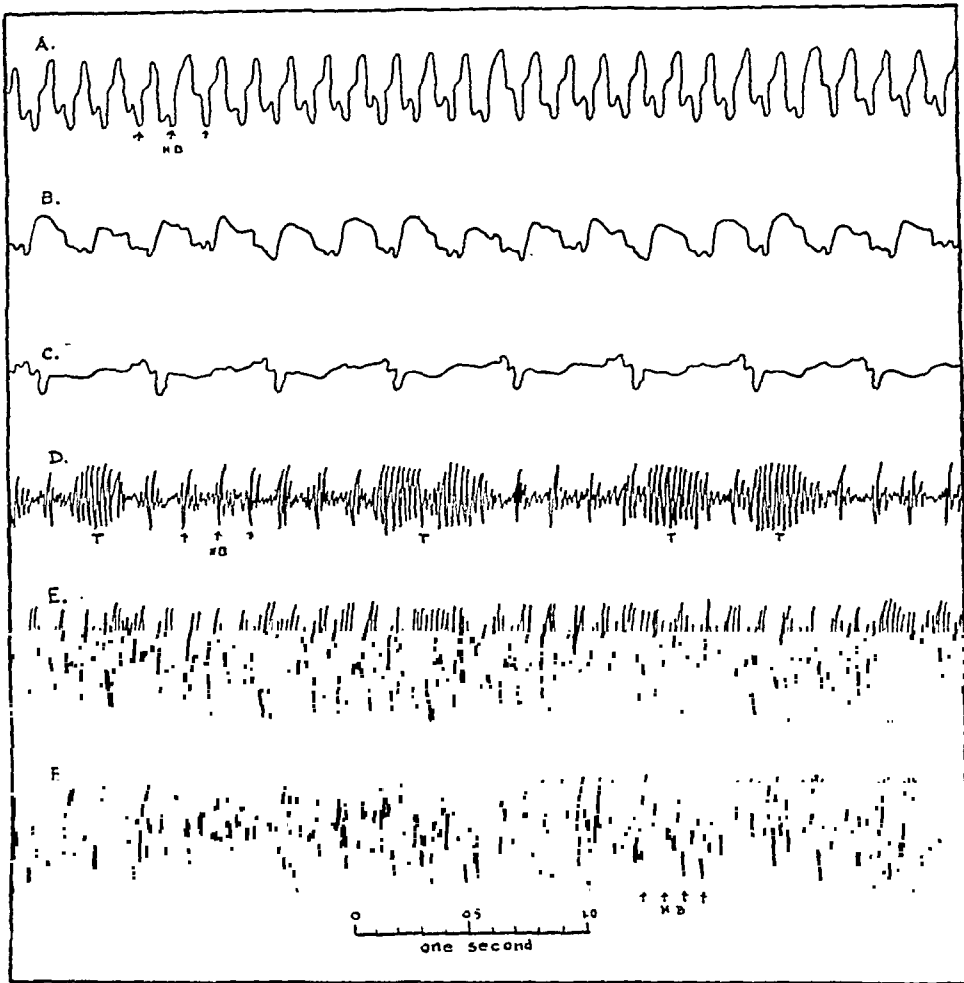


Fig. 1. Vibrograms of nestling house wrens illustrating the effect of decreasing air temperature on recently hatched (0-day), poikilothermic nestlings as compared with 12-day, homoiothermic nestlings. Heart beat (HB) and muscle tremors (T) are indicated. A, B, C, 0-day nestlings at air temperatures of 95°, 80°, 70°F. (35°, 26.7°, 21.1°C.) respectively. Heart rates are 404, 227, and 116 per minute. D, E, F, 12-day nestlings at 95°, 80°, 70°F. respectively; heart rates are 418, 520, and 690 per minute. Note: The difference in the form of the tracings is due to difference in position of birds on the pickup crystal; thus, newly hatched nestlings rest on their chests producing a record similar to "apex beat" of human cardiology.

As would be expected of a cold-blooded animal the rate of heart beat as well as the amplitude decreased as the temperature dropped. In 12-day nestlings (fig. 1, D, E, F), however, the heart rate increased markedly with the same drop in air temperature as would be expected in a warm-blooded animal. Further-

more, pronounced tremors were present which increased with the decrease in air temperature until at 70°F. tremors were nearly continuous and almost obscured completely the recording of the heart beat (fig. 1 F). In striking contrast, no such coördinated tremors were detected in the cold-blooded nestlings; thus, in figure 1 A, B, C, the pen line is smooth, no high frequency (relatively) vibrations interfering with the recording of the heart movements.

The way in which tremors first developed is interesting and perhaps significant. At higher temperatures tremors, if present at all, were often periodic occurring in short periods, sometimes less than a second in duration, alternating with longer periods in which no tremors were recorded (fig. 1 D). With a decrease in air temperature these tremor periods increased in frequency and duration until at 70°F. tremors were continuous in 9, 12 and 15 day old birds. Even then, however, the frequency and intensity of the recorded vibrations continued to vary periodically (fig. 1 F). Tremors appear to develop in ontogeny in much the same way often being restricted to short periods when first detected between 3 and 6 days of age and becoming more pronounced at 9, 12 and 15 days. In the chickadee, which is very similar to the wren in its developmental history, tremors were first detected at 4 days after hatching which coincided with the first appreciable rise of the body temperature above that of the environment. With most 4-day chickadees tested at 70° definitely recognizable tremors were recorded. These were usually periodic, sporadic, or irregular in occurrence and of low frequency and intensity. The body temperature at this age averaged 2° to 4°F. above 70°F. In most 3-day nestlings, on the other hand, recognizable tremors could not be picked up by the apparatus, and the body temperature was equal to or only slightly above that of the environment. Tremors probably develop more gradually than this would seem to indicate, but the coördination of tremors throughout the body (thus setting the entire body into rhythmic vibration and producing an unmistakable graphic record) seem to occur coincident with the first indications of the development of temperature control.

The relation between tremors and body temperature of wrens of different ages at an air temperature of 70°F. (21.1°C.) is shown in figure 2. A curve for feather development is also included. Counts of the number of penstrokes (on graphic records) definitely resulting from tremor movement were taken as a means of quantitative measurement of tremors. Samples were taken at 5-second intervals over many feet of records so that periods without tremors would be averaged in with tremor periods in proportion to their occurrence. Thus, both the duration (ranging from 0 to 100 per cent) and the actual frequency of the coördinated "firing" of muscle units during active periods (usually ranging between 30 and 40 per second) were taken into account in the average figures plotted in figure 2.

Apparently, the control of heat loss as indicated by the feather development lags behind the development of the muscle tremor heat production mechanism. Thus, at 9 days of age tremors were vigorous but feather development poor and body temperature low (at standard conditions). At 15 days feather development more than doubled and the body temperature was maintained higher

with about the same amount of tremors. Finally, the normal body temperature of the adult is maintained with few or no tremors at this particular temperature. The curve of standard heart rate is roughly similar to that of muscle tremors; heart rate increases to 12 days then decreases in the adult (Odum, 2).

Increase in tremors in nestlings with a decrease in air temperature often occurred with little increase in general activity (i.e., actual movements) although this was not measured quantitatively over long periods. When movements did occur during the recording periods it was very noticeable that tremors stopped or slowed down immediately after and sometimes just before the movement. Accordingly, tremors and movements operate in a complementary

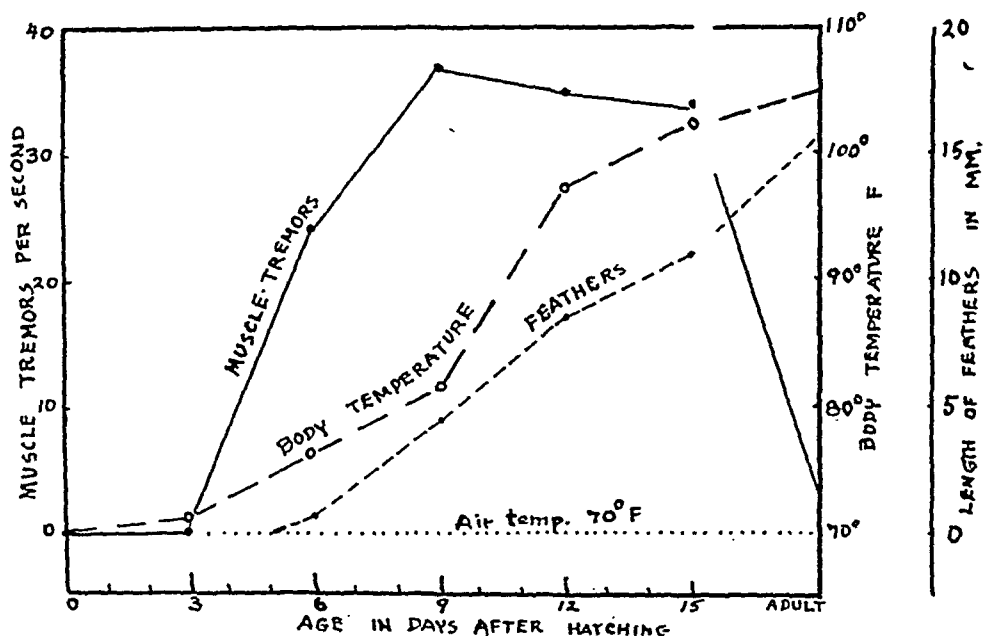


Fig. 2. Relation of muscle tremors, body temperature and feather development in house wrens. See text for further explanation. Body temperature data are from Kendeigh (4) and feather data from Boulton (5).

manner. An anticipatory stopping of tremors preceding a movement was especially striking in records of adult birds incubating on their nests (Odum, 2).

In preliminary experiments on precocial birds, in which temperature control begins to develop before hatching, what appear to be brief tremor periods (producing a record similar to fig. 1 D) have been detected as early as 9 days' incubation in the case of unopened eggs of the ring-necked pheasant at 99°F. (37.2°C.). According to Romanoff (6) this is approximately the period in the development of the domestic fowl when embryo temperature begins to rise above incubation temperature.

**DISCUSSION.** In making quantitative measurements of these total body tremors from records such as shown in figure 1 a number of variables need to be considered if a complete picture of the response to temperature changes is obtained. As already indicated the relative duration as well as the frequency of



tremors is important. In 12 day wrens, for example, tremors were recorded 12 per cent of the time at 95°, 93 per cent at 80°, and 100 per cent (shivering continually) at 70°F. The frequency of tremors in these small birds is apparently upwards to 30 or 40 per second, considerably more rapid than the shivering rate reported for man (7). Activity as mentioned above is another factor effecting tremors. Still another variable is the amplitude or intensity of the individual tremor contractions. Apparently amplitude increases with an increase in duration and frequency, but this could not be measured quantitatively with the setup used.

These various muscle adjustments produce a very sensitive mechanism in these small birds which seems to be especially responsive at temperatures below thermal neutrality (about 100°F. or 37.8°C. in homoiothermic nestlings). Within the temperature range which we have considered variations in tremors and other muscular activity seem adequate to account for the observed variations in heart rate and metabolic rate. However, at other temperatures or under other conditions different mechanisms might come into play. As Dworkin (7) has pointed out temperature regulation as a whole is probably complex involving many factors and mechanisms, efficient regulation depending on the coördination of various factors by centers in the central nervous system.

#### SUMMARY

The development of coördinated muscle tremors, such as are picked up and recorded by the cardio-vibrometer, corresponds closely with the development of temperature regulation in the small altricial species,—the house wren and black-capped chickadee. No tremors were recorded from newly hatched and 3-day nestlings which are poikilothermic but were present at all later ages roughly corresponding to the development of homoiothermy and inversely related to the air temperature. At 95°F. (35°C.) (and also at first appearance in ontogeny) tremors were often periodic in occurrence; with a decrease in temperature tremor periods tended to increase in duration and frequency until shivering became continuous. During active periods the frequency of tremors (as indicated by total body vibrations) was 30 to 40 per second. The muscle tremor heat production mechanism apparently develops more rapidly at first than does the control of heat loss as indicated by feather growth. In the precocial pheasant periodic tremors were first detected at 9 days of incubation in the unopened egg at incubation temperature.

The writer is indebted to Dr. S. C. Kendeigh under whose direction these experiments were begun and to the officers of the Edmund Niles Huyck Preserve, Rensselaerville, N. Y., where experiments with the chickadees were carried out.

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# SPEED OF RESPONSES OF VARIOUS MUSCLES OF CATS

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In a recent paper Rosenblueth, Wills and Hoagland (1941) reported that the sartorius and soleus muscles of cats, when stimulated indirectly, could show two crests in both electrogram and mechanogram. The temporal properties of the two components of soleus were different from those of sartorius. The present work was undertaken to examine this phenomenon in a greater variety of muscles, and also to see whether different nerve-muscle systems display the phasic activity of muscle described by Rosenblueth and Cannon (1940) and Cannon and Rosenblueth (1940). The latter point was studied with reference to a possible parallelism between the speed of a muscle and its properties as regards the several "stages of transmission."

**METHODS.** Seven muscles were used: diaphragm (Head's slip), gastrocnemius, inferior oblique of eye, plantaris, sartorius, solcus and tibialis anticus. In most cases the cat, anesthetized with dial (Ciba), was the animal used, but in a few of the diaphragm experiments rabbits anesthetized with urethane were the subjects.

For the study of single twitches the muscles were attached to a torsion spring myograph of the Sherrington type. The contractions were isometric. The diaphragm was studied semi-isometrically because of inability to devise any satisfactory method for strict isometric recording from Head's slip.

During prolonged stimulation for registering the phasic properties of the nerve-muscle system, the activity of the muscle was recorded by a writing lever pulling against rubber bands. The magnification was about 10-fold.

Electrical records were made from the muscles simultaneously with the single twitch except for diaphragm and inferior oblique. The leads were silver needles, chlorided when a direct-coupled amplifier was used. The muscle was crushed about 5 mm. below the tie connecting it with the myograph, and one needle was inserted into the tissue between the tie and the crush. The other lead was in normal muscle.

In most of the experiments a capacity-coupled amplifier with relatively small coupling condenser was used, but in a few cases a direct-coupled amplifier was employed. The amplified responses were led to a cathode-ray oscillograph. The image of the oscilloscope screen was photographed on film simultaneously with the myograph tracing, obtained by sending the beam of reflected light from the mirror of the myograph to the back of the film in the camera.

Spread-out records of the spike potentials of the various muscles were obtained by making single standing photographs with the sweep of the cathode-ray

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tube at high speed. The sweep was tripped by the stimulus, so that the records start with the shock artifact. The speed of the sweep was calibrated with known sine-wave frequencies.

The stimulating electrodes were shielded silver wires applied to the motor nerve, crushed or cut centrally. The stimuli were condenser shocks rendered diphasic by passage through a transformer. The frequency of stimulation was regulated by a gas-discharge tube (type 885).

RESULTS. Figure 1 shows the simultaneous isometric mechanograms and monophasic electrograms of the various muscles. It will be seen that several, but not all, of the muscles had two peaks in their mechanograms. The times required for the muscles to produce the various tension peaks can be determined

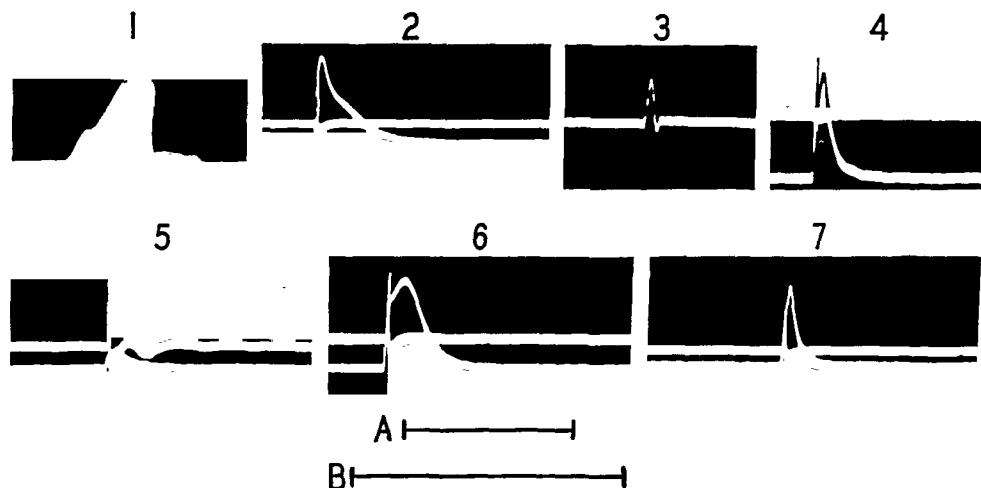


Fig. 1. Simultaneous isometric mechanograms (lower tracings) and monophasic electrograms (upper tracings) of muscles. In the cases of diaphragm and inferior oblique only the myogram is shown. The myogram of diaphragm was not strictly isometric. 1—diaphragm, 2—gastrocnemius, 3—inferior oblique, 4—plantaris, 5—sartorius, 6—soleus and 7—tibialis anticus. Calibrations: A, 1 sec., for diaphragm. B, 1 sec., for all other muscles.

from these records by use of the calibrations for speed of the recording surface. Such values appear in table 1.

Figure 2 consists of the spread-out spikes of the action potential of the various muscles. The lower part of each panel is the calibration of the speed of the sweep. Here also some records have two or more peaks, and some have only one. The times from the beginnings of the action potentials to the various peaks appear in table 1, along with the corresponding times for the mechanograms.

The muscles in table 1 have been arranged vertically according to increasing speed of reaching the major peak of tension. The average times from the beginning of contraction to maximal tension were: diaphragm 480 msec., soleus 77.7 msec., sartorius 28.7 msec., plantaris 27.4 msec., tibialis anticus 24.3 msec., gastrocnemius 22.5 msec., and inferior oblique 18.7 msec.

TABLE 1

*Average times to various peaks of mechanogram and electrogram of single twitch*  
 All peaks were not present in all experiments

MUSCLE	RECORD	MSEC. TO PEAKS		
		# 1	# 2	# 3
Diaphragm.....	Myogram	172 (10)	518 (10)	
	Electrogram	4.1 (2)	15.6 (2)	
Soleus.....	Myogram	35.8 (3)	103 (6)	
	Electrogram	2.3 (6)	4.6 (6)	6.5 (1)
Sartorius.....	Myogram	28.7 (7)	76.6 (5)	
	Electrogram	3.4 (6)	5.3 (3)	
Plantaris .....	Myogram	27.4 (2)		
	Electrogram	2.8 (2)	4.3 (2)	
Tibialis anticus.....	Myogram	24.3 (6)		
	Electrogram	2.3 (6)	4.2 (6)	7.0 (3)
Gastrocnemius.....	Myogram	22.5 (2)		
	Electrogram	2.1 (2)	4.6 (1)	
Inferior oblique.....	Myogram	18.7 (3)		
	Electrogram	1.3 (2)	1.8 (2)	

Parentetical figures represent the numbers of animals giving the responses averaged to obtain the mean values given here.

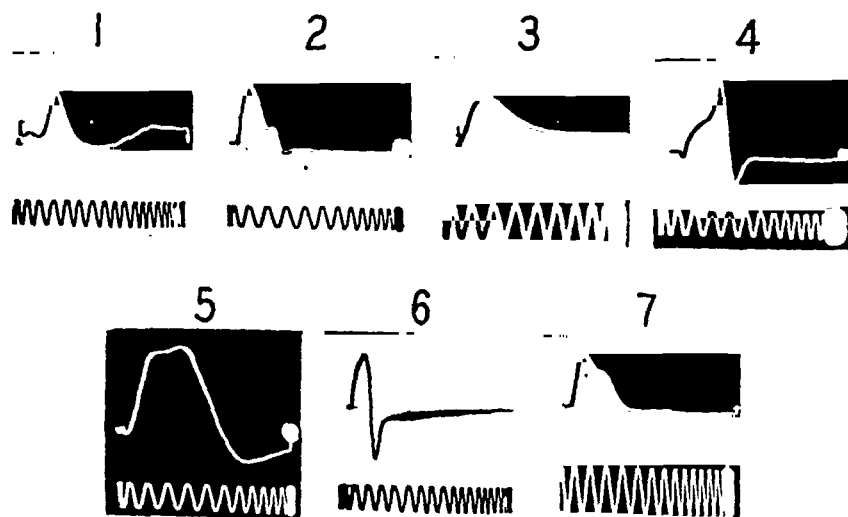


Fig. 2. Spread-out spikes of action potentials of muscles. 1—diaphragm, 2—gastrocnemius, 3—inferior oblique, 4—plantaris, 5—sartorius, 6—soleus and 7—tibialis anticus. Calibrations (in cycles per sec.): 1, 500; 2, 500; 3, 1000; 4, 500; 5, 500; 6, 200; 7, 500.

It will be noted that the three slowest muscles (diaphragm, soleus and sartorius) have two peaks of tension in the mechanogram. In no case, however, do either the fast or the slow components have the same properties in different muscles. This supports the previous conclusion (Rosenblueth, Wills and Hoagland, 1941) that while various muscles may be composed of a mixture of slow and fast fibers, these elements have different properties in different muscles.

The order of speed in reaching the principal peak in the electrical record was not the same as for the mechanogram. The mean times for development of maximal negativity were: sartorius 4.5 msec., diaphragm 4.1 msec., soleus 3.9 msec., plantaris 3.7 msec., tibialis anticus 3.4 msec., gastrocnemius 2.0 msec., and inferior oblique 1.6 msec. While the agreement between the orders of reaching the principal peaks in the mechanogram and electrogram respectively is not good in detail, there is a general tendency for the faster muscles to require less time for development of maximal negativity. Sartorius seems to be the principal atypical muscle in this regard.

TABLE 2  
*Average frequencies of stimulation for appearance of early "stages"*

MUSCLE	NUMBER OF EXPERIMENTS	FREQUENCY FOR APPEARANCE OF:		
		Tetanus	Stage 2	Best sequence: 1, 2, 3a, 3b, 3c
Diaphragm.....	5	36	350	500
Soleus.....	4	22	225	340
Sartorius.....	4	23	275	465
Plantaris.....	5	25	280	430
Tibialis anticus.....	9	33	300	465
Gastrocnemius.....	7	31	250	400
Inferior oblique.....	6	69	700	1000

Table 2 gives the average values of the frequencies of stimulation of motor nerves necessary for the appearance in the corresponding muscles of tetanus, stage 2 and the clearest sequence of stages 1, 2, 3a, 3b and 3c, the muscles being arranged vertically in the table in order of increasing speed of contraction. The nomenclature of the "stages of transmission" used here is that of Rosenblueth and Cannon (1940).

It is evident that there was a general, but not very exact, parallelism between the speeds of contraction of the various muscles and the frequencies given in table 2. Diaphragm is the most striking exception to this generalization: while having a quite slow speed of contraction (table 1), it requires some of the higher frequencies of nerve stimulation for appearance of the various stages of contraction. The parallelism between the frequencies required for appearance of tetanus and of the other early stages is good, but not perfect.

A study of the late stages (4 and 5) has been made with respect to their relative tension productions in per cent of the tension of stage 1. The data are presented in the form of averages in table 3. This table contains also the mean

times elapsed from the beginning of nerve stimulation to the appearance of stage 5 for different frequencies of stimulation, and the frequencies of appearance of stage 5 in the various nerve-muscle systems.

It can be seen from table 3 that the 5th stage of neuromuscular transmission was obtained in all the systems studied in this work, but most consistently in soleus, plantaris and gastrocnemius. In sartorius, tibialis anticus and inferior oblique the 5th stage was obtained in about one half the experiments, while in

TABLE 3  
*The late "stages of neuromuscular transmission" in various muscles*

MUSCLE	STIM. FREQ.	NO. OF EXPTS.	NUMBER OF 5TH STAGES	MINS. TO 5TH STAGE	4TH STAGE IN PER CENT OF 1ST	5TH STAGE IN PER CENT OF 1ST
Diaphragm.....	60	6	1	130	38.0	77.0
	120	1	0			
Soleus.....	60	3	3	63	7.1	40.9
	75	2	2	10	1.9	27.2
	120	1	1	4	10.3	66.6
Sartorius.....	60	4	3	67	3.2	19.5
	90	1	0			
Plantaris.....	60	3	3	71	9.6	26.4
	75	3	3	26	10.1	22.8
	90	1	1	30	11.0	22.0
	120	1	1	4	22.0	33.0
Tibialis anticus.....	60	7	3	53	1.2	7.7
	75	1	1	6	2.4	8.9
	85	2	1	10	0.4	1.4
	100	2	1	6	0.0	2.0
Gastrocnemius.....	60	6	6	83	6.7	23.9
	75	1	1	18	12.8	44.2
	120	1	1	10	7.2	31.1
Inferior Oblique.....	60	1	0			
	75	1	1	60	9.6	52.0
	120	1	0			
	150	1	1	10	15.3	18.4

diaphragm it appeared only once in 7 experiments. These results, in conjunction with those of table 2, allow us to conclude that the sequence of the several stages of transmission is quite general in nerve-muscle systems.

Maltesos and Weigman (1939) have found that stimulation of the chorda tympani at 425 cycles per sec. produced secretion by the submaxillary gland of a phasic nature, reminiscent of the sequence 1, 2, 3a, 3b and 3c in a nerve-muscle preparation. Wills (1941) has reported that chorda stimulation at frequencies

of 28.6 to 60.0 per sec. gave a series of changes of rate of secretion by the submaxillary gland analogous to the sequence of "stages" seen in a nerve-muscle preparation at relatively low frequencies (Rosenblueth and Morison, 1937; Rosenblueth and Luco, 1939). This evidence might suggest that phasic response to stimulation is a general property of nerve-effector systems, and not peculiar to nerve-muscle preparations. Lanari and Rosenblueth (1939), however, found no evidence of phasic changes of transmission in the nictitating membrane when the sympathetic postganglionic nerve supply was stimulated at various frequencies.

Table 3 shows that there was a tendency for the slower muscles to have, during stages 4 and 5, larger residues of the initial tension than the faster ones had. Gastrocnemius was the chief exception to this generalization, during stages 4 and 5 having greater tensions in per cent of that of stage 1 than might be expected from its position in the table. It is also apparent from table 3 that, in all cases, increasing the frequency of stimulation brought about earlier appearance of stage 5, as had been reported previously by Rosenblueth and Luco (1939).

#### SUMMARY

The number of peaks of negativity found in the monophasic action potential of muscle seems to have no definite relation to the number of peaks of tension appearing in the myogram (figs. 1 and 2; table 1).

While various muscles seem to be composed of a mixture of slow and fast elements, these elements in different muscles have different properties. Thus, there is no typical slow or fast fiber (table 1).

There is a general parallelism between the speed of contraction of a muscle and the frequency of motor nerve stimulation needed for the appearance of the various stages of neuromuscular transmission (table 2). In the late stages there is a tendency for the faster muscles to develop less tension in per cent of that during stage 1 than the slower muscles produced (table 3).

The sequence of stages of transmission seems to be general in striated nerve-muscle systems.

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# ACCOMMODATION IN MAMMALIAN MOTOR NERVES

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The term "accommodation" (Nernst, 1908) is used to describe a rise of the threshold of a nerve fiber when an electric current passes through it. This rise of threshold explains why the response of a nerve to a prolonged application of direct current may be a single impulse. A rise of threshold also explains why slowly increasing currents may fail to stimulate even though the final intensity reaches a value many times greater than the rheobase.

Following Hill's (1936) theoretical suggestions, Soldant (1936a, b) measured the time constant of accommodation of several nerves and found values of 42 to 83 msec. for mammalian motor nerves. These figures imply that progressively increasing currents which do not attain the value of 1 to several (n) rheobases within 1 to n times the time constant (about 60 msec.) will fail to stimulate. In other words, a current which increases progressively to an intensity 10 times greater than the threshold for suddenly applied direct current should reach that value within less than about 0.6 sec. in order to stimulate. A further implication is that if a constant current of intensity 10 times the rheobase is applied to a nerve, repetitive discharges should occur for less than 0.6 sec.

Rosenblueth (1940) frequently observed repetitive discharges for as long as 15 sec. or more when cats' motor nerves were treated with direct currents only 3 to 6 times stronger than the threshold. The discrepancy between these observations and Soldant's (1936a) measurements, interpreted on the basis of Hill's (1936) theory of accommodation, led to the present study.

**METHOD.** In cats, under dial anesthesia (Ciba, 0.7 cc. per kgm. intraperitoneally) a tracheal cannula was inserted. An Achilles tendon was dissected and tied, and the region of the calcaneum where it inserts was cut. The thread on the tendon was attached to a tension myograph pulling against rubber bands and recording the contractions of the muscles on a kymograph. The muscles were used as indicators of activity of the motor fibers in the popliteal nerve. The leg was fixed by means of drills inserted into the tibia.

The sciatic nerve was cut as high in the hip as possible. The hamstring nerves were cut and dissected away from the sciatic. The cut peripheral end of the nerve was tied and the trunk was dissected for about 2 cm., to the level of an arteriole and a venule which join the nerve about 0.5 cm. below the trochanter. These vessels were carefully preserved and, in addition, throughout the dissection the vessels of the nerve were respected. The importance of the circulation will be emphasized later. The segment of nerve dissected as above was sometimes found inadequately supplied with blood. The dissection was then con-

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tinued peripherally until a satisfactory stretch of nerve was found. Experience showed that consistent and stable results were obtained if, and only if, the two arterioles which run between the peroneal and the popliteal nerves were visibly carrying blood after the central end of the sciatic was tied and the stretch was freed that was to be introduced into the glass support of the electrodes.

The electrodes were placed as follows. The nerve was threaded into a glass tubing about 3.5 cm. long, supported by a glass rod held by a clamp. A sopper fixed the thread tied to the nerve and also prevented desiccation. The glass tubing had two short, open side-branches, with a distance of 1.5 cm. between them. Through these side-branches were introduced the wicks, soaked with Ringer, which connected the sciatic with the impolarizable electrodes. The wick distal to the muscle was in contact with the damaged region of the nerve between the cut and the tie. The other wick was in contact with normal tissue.

Two sets of electrodes were used, with similar results. Some were large calo-

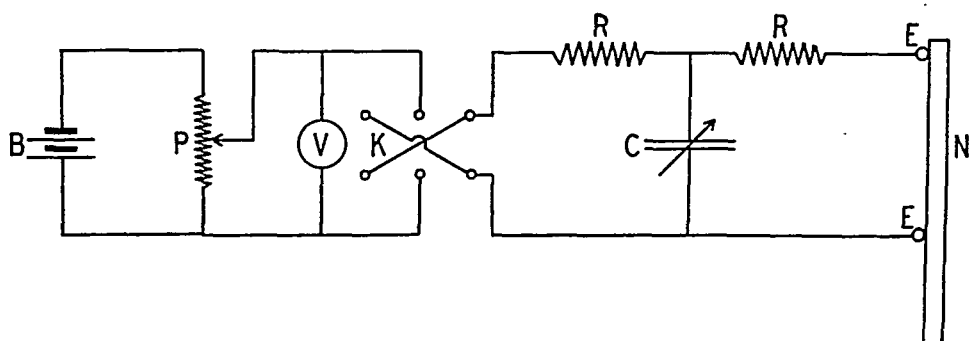


Fig. 1. Diagram of the stimulating circuit. *B*: 22.5 to 45 v. battery. *P*: 50,000  $\omega$  potentiometer. *V*: voltmeter. *K*: reversing key. *R*: 100,000  $\omega$ . *C*: variable capacity, 0 to 30  $\mu$  F. *E*: impolarizable Hg-HgCl or Ag-AgCl electrodes. *N*: nerve.

mel half-cells, the others large chlorided silver plates. The bridge from these electrodes to the nerve was first a short column of agar-Ringer, then the wicks wet with Ringer, mentioned above. The impolarizability of the electrodes within the range of currents employed in the experiments was tested by reading the current corresponding to different voltages from an ammeter in series with the electrodes and nerve. The system was found to obey Ohm's law accurately, thus showing that no counter e.m.f. of polarization developed at the electrodes with the highest voltages employed.

Exponentially increasing currents were obtained by the circuit in figure 1. The resistance of the electrodes, repeatedly measured, was 10,000  $\omega$ ; that of the nerves, also repeatedly measured, varied from 3,000 to 5,000  $\omega$ . The total effective resistance of the circuit, through which the shunt capacity was charged, varied with the resistance of the nerve and with the different settings of the potentiometer. This variation was only slight, however, the extremes being 53,000 and 55,000  $\omega$ . No significant error was made, therefore, by using the average resistance of 54,000  $\omega$  in all instances for the calculation of the time con-

stant of the exponential rise of the currents applied. The time constant (in microseconds) was equal to the capacity (in microfarads) times 54,000.

The currents were sent through the nerve first in a descending direction (cathode towards the muscle), then in the opposite direction. The results are mainly concerned with the action of descending currents.

Hyperventilation was produced by connecting an artificial respiration pump with the tracheal cannula and by increasing the positive pressure of the pump to the desired degree, without changing the rate. Hypercapnia was obtained by delivering a moderate continuous stream of  $\text{CO}_2$  through a narrow glass tubing inserted into the tracheal cannula. The animal breathed spontaneously in these cases. It promptly developed polypnea, but the consistent experimental results showed that a steady equilibrium was soon reached and that the  $\text{CO}_2$  pressure in the arterial blood was increased.

**RESULTS.** A. *Accommodation in normal nerves.* The currents passing through the nerve in the circuit of figure 1 rise exponentially with a time constant determined by the capacity to a peak value proportional to the voltage indicated by the voltmeter. When currents of progressively slower rate of exponential rise are tested the peak voltage for threshold excitation increases gradually. This increase may be interpreted as due to the rise of threshold during the passage of the current. The curves correlating the time constant of the rising currents with the corresponding peak voltage necessary for stimulation may thus be used for the study of accommodation (see Hill, 1936). The experimental procedure was as follows.

The threshold for direct current, which is equivalent to an instantaneous exponential rise, or to a time constant 0, was first determined for a response of small amplitude. A pointer writing a horizontal line on the kymograph was adjusted to mark the amplitude of response selected. The variable capacity of figure 1 was then set to give different time constants for the currents and the corresponding threshold voltages were determined, maintaining the amplitude of response fixed. The time constants were first progressively increased, then decreased, and finally varied at random. In each observation, as mentioned under Method, a descending current was followed by an ascending one, but the measurements were made for the descending stimuli.

In some preparations a progressive decrease of excitability of the nerve was apparent, whether the tests were made with currents of a single or of different time constants. Experience showed that this progressive deterioration of the nerve was due to deficient blood supply. By shifting the electrodes, some region of the nerves was always found where the threshold for any given current did not vary for over an hour, the time necessary for the construction of a peak voltage-time constant curve. Only the observations made under this stable condition will be considered.

The peak voltage-time constant curves of normal nerves were of two different types. Figure 2A illustrates a characteristic example of the first type. The data from which this curve was constructed are given in table 1a. The voltmeter could be read with a limit of error of  $\pm 0.03$  v. A change of 0.05 v. in

the currents was readily measurable in the amplitude of the responses evoked. The accuracy of this and the following curves is therefore within about 2 per cent for the small time constants, and better than that for the higher points.

In contrast with the smooth curve of figure 2A the upper curve of figure 3A (table 1b) exhibits a break. This was typical of the second type of curves encountered. The break separated segments of curves corresponding to different processes, as indicated by differences in the responses to stimuli belonging to one or the other segment. Figures 4A and C illustrate these differences. All the responses caused by stimuli which plotted in the lower segment of the curves

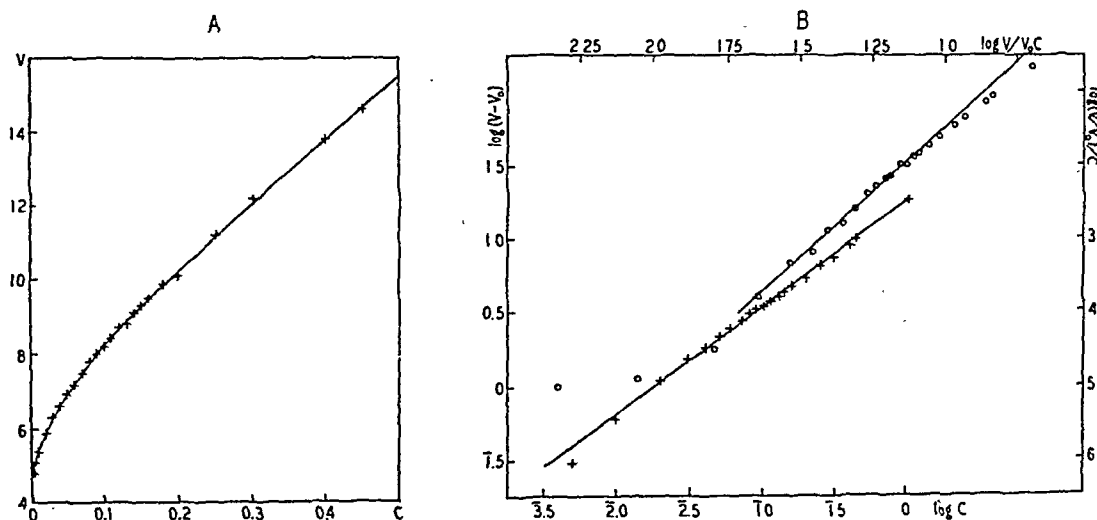


Fig. 2A. Normal peak voltage-time constant curve. Ordinates: voltage readings on voltmeter in figure 1; this value is lineally proportional to the peak value of current through the nerve, approached exponentially; the peak current may be obtained by dividing the voltage by 220,000. Abscissae: shunt capacity (fig. 1) in  $\mu\text{F}$ ; the time constant (in msec.) of the exponential rise of current through the nerve may be obtained by multiplying the capacity (in  $\mu\text{F}$ ) by 54. The curve indicates what peak voltage was necessary for threshold stimulation (constant small muscular response) at the corresponding time constant. The order in which the observations were made is listed in table 1a.

B. Crosses (lower scale of abscissae and left scale of ordinates): test of the points in A for a parabola (see p. 634). Circles (upper and right-hand scales): test for Hill's (1936) formula (see p. 639).

were brief, as in figure 4A; all those evoked by currents which plotted in the second segment were prolonged, as in figure 4C. At or near the point at which the break in the curves took place it was possible sometimes to observe double responses, a brief contraction followed by a sustained development of tension (fig. 4B).

The records in figure 5 illustrate the responses to suprathreshold currents of constant peak voltage and variable time constant of rise. It is noticeable that as the time constant increases the decrease of response is mainly at the expense of the early sharp peak elicited by instantaneously rising direct current (fig. 5, 1st response) rather than at the expense of the later relatively sustained tension.

These records may be interpreted on the assumption that the response to direct current involves an early component, which accommodates rapidly, and a later component, which accommodates more slowly. With the slowly rising currents the first component is absent.

The break in the curves of the second type was often quite obvious, as in figure 3A (crosses); it was sometimes not readily detectable (see fig. 8A, circles). The change in the type of response, brief or sustained, evoked by different currents,

TABLE 1

*Data from which were constructed the curves in figures 2A (curve a), 3A crosses (curve b), 3A circles (curve c), 8A crosses (curve d), 8A circles (curve e), 9A crosses (curve f), and 9A circles (curve g)*

The table shows the order in which the observations were made

CURVE A		CURVE B		CURVE C		CURVE D		CURVE E		CURVE F		CURVE G	
c	v	c	v	c	v	c	v	c	v	c	v	c	v
0	4.8	0	3.0	0	1.5	0	2.5	0	1.15	0	1.9	0	4.2
0.1	8.2	0.1	3.8	0.1	2.0	0.1	4.8	0.25	3.9	0.15	4.5	0.15	8.4
0.07	7.5	0.2	4.4	0.2	2.3	0.2	6.2	0.35	4.4	0.05	3.1	0.1	7.4
0.03	6.3	0.4	5.5	0.4	2.75	0.3	7.3	0.45	4.8	0.25	5.3	0.2	9.3
0.02	5.9	0.05	3.45	0.8	3.4	0.4	8.2	0.55	5.2	0.35	6.2	0.3	10.7
0.01	5.4	0	2.9	0.3	2.5	0.5	9.3	0.65	5.4	0.3	5.7	0.05	5.9
0.005	5.1	0.6	6.3	0.6	3.15	0.35	7.8	0.75	5.6	0.2	4.9	0.4	12.0
0	4.8	0.9	7.5	1.0	3.6	0.25	6.8	0.5	5.0	0.1	3.8	0.35	11.4
0.05	6.95	0.15	4.1	1.5	3.8	0.15	5.6	0.4	4.5	0.02	2.4	0.25	9.8
0.08	7.8	0.3	4.9	3.0	4.0	0.05	4.1	0.3	4.1	0	1.75	0.13	7.8
0.11	8.4	0.5	5.9	4.5	4.15	0.02	3.3	0.2	3.4	0.4	6.4	0.08	6.7
0.13	8.8	0.8	7.2	3.7	4.1	0.6	10.0	0.1	2.6	0.08	3.5	0.03	5.1
0.16	9.5	1.2	8.6	2.2	3.9	0.7	10.8	0.05	2.15	0	1.75	0	3.8
0.06	7.2	1.5	9.2	1.2	3.75	0.45	8.7	0	1.1				
0.04	6.6	0.7	6.75	0.5	3.0	0.55	9.6	0.15	3.1				
0.2	10.1	2.0	9.6			0.9	12.0	0.25	3.7				
0.25	11.2	3.0	10.2					0.9	5.7				
0.09	8.0	1.0	8.0					1.0	5.75				
0.3	12.2	0.25	4.7					0.02	1.65				
0.4	13.8	4.0	10.6										
0.14	9.1	0	3.2										
0.45	14.8												
0	4.9												
1.0	23.5												
0.18	9.9												

however, was always striking. That the curves had two segments whenever the two types of response were encountered was shown by a break in the linear test of the curves, which will be described later (p. 640).

The position of the break in the curves varied for different animals. It sometimes was present at short time constants (fig. 3A), while in other cases it did not become apparent unless quite long-time constants were tested (up to 0.15 sec.;  $3\mu F$ ). In nerves which yielded smooth curves similar to that in figure 2A,

if currents were applied with such long time constants, sustained responses were invariably obtained—i.e., the break in the curve was revealed. It may be inferred, therefore, that a break in the curves is a general phenomenon, not the occasional property of some nerves. In some cases, however, the second segment may only be seen with very slowly rising currents which require a correspondingly high voltage for stimulation. Such high voltages—i.e., over 20 v. (more than 0.1 mamp.)—may cause irreversible or very slowly reversible changes in nerve. The curves were carried out to points which did not require unduly high currents; for this reason some do not show the break.

In some experiments the resistances of 100,000  $\omega$ , usually employed, were changed to 250,000  $\omega$ , thus multiplying the effective resistance of the circuit by 2.5. Observations were made with capacities as large as 30  $\mu$ F. A time con-

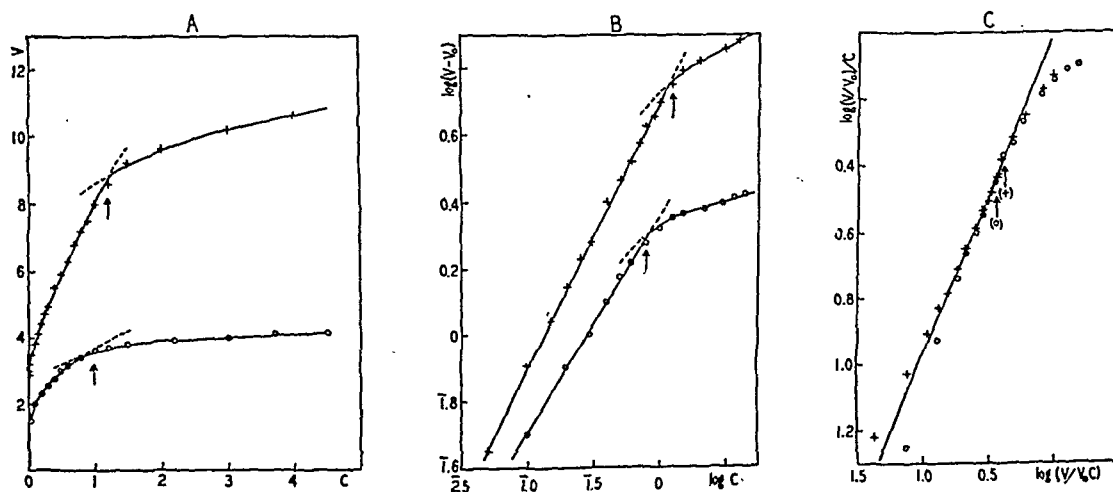


Fig. 3A. Peak voltage-time constant curves before (crosses, table 1b) and during (circles, table 1c) a prolonged period of hyperventilation. The arrows indicate the time constant at which the type of response recorded changed: to the left of the arrows the muscular responses were brief twitches; to the right, the responses were relatively sustained tetani.

B and C. Tests of the curves in A for a parabola and for Hill's formula, respectively.

stant of 4 sec. was thus tested. In all cases responses were obtained with peak voltages no greater than about 10 times the rheobase, i.e., less than 0.1 mamp. The responses could have latencies as long as 6 sec. and were all of the sustained type. Although, as mentioned above, intense currents were found to produce slowly reversible changes of excitability, the responses elicited by these slowly rising currents could be repeatedly obtained with only a slight progressive intensification of the peak voltage. Figure 4D illustrates a typical delayed and sustained contraction caused by a current reaching a peak intensity of 0.08 mamp., with a time constant of 3.9 sec.

B. *Hyperventilation.* Hyperventilation, with the consequent acapnia, promptly resulted in marked changes of electrical excitability and accommodation of nerve. In figure 6A is illustrated the change of rheobase. As soon as hyperventilation was started the rheobase decreased, as evidenced by the incre-

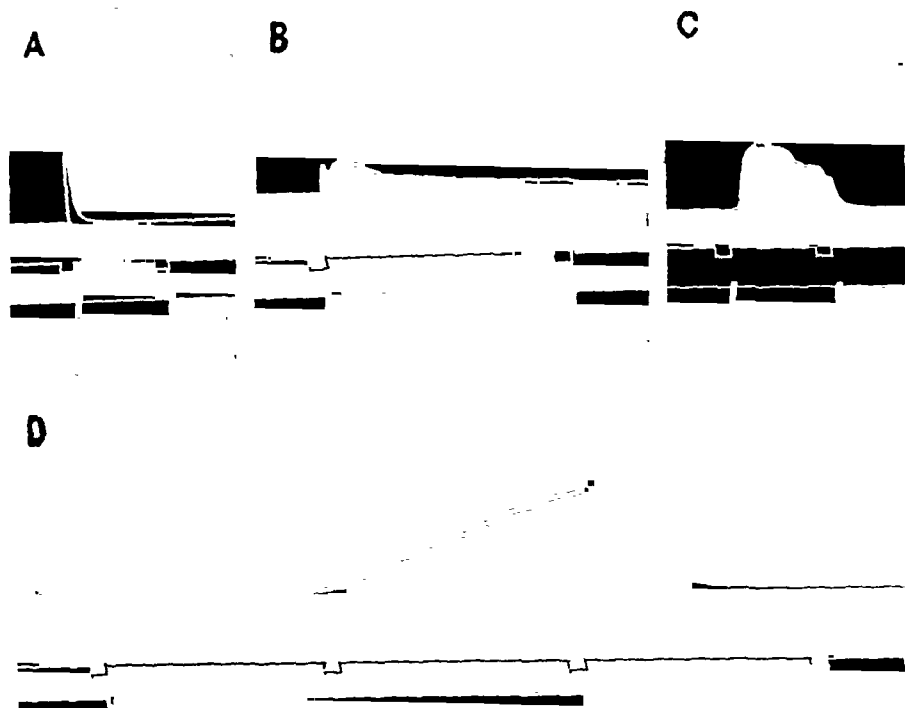


Fig. 4. Different types of muscular responses elicited by currents of progressively slower rise. Contractions of the Achilles tendon muscles. Time signal; for A and C, 2 sec.; for B and D, 5 sec.; the speed of the kymograph was the same for all records. The currents were applied between the lower signals.

A. Time constant of the exponential increase of the current: 27 msec. Asymptotic voltage indicated by the voltmeter in figure 1: 15 v.

B. Time constant: 43.2 msec. Voltage: 20.

C. Time constant: 162 msec. Voltage: 25.

D. In another animal. The 2 resistances  $R$  in the circuit of figure 1 were increased to 250,000  $\omega$  each. Time constant: 3,900 msec. Voltage: 40. Intensity: 0.08 mamp.

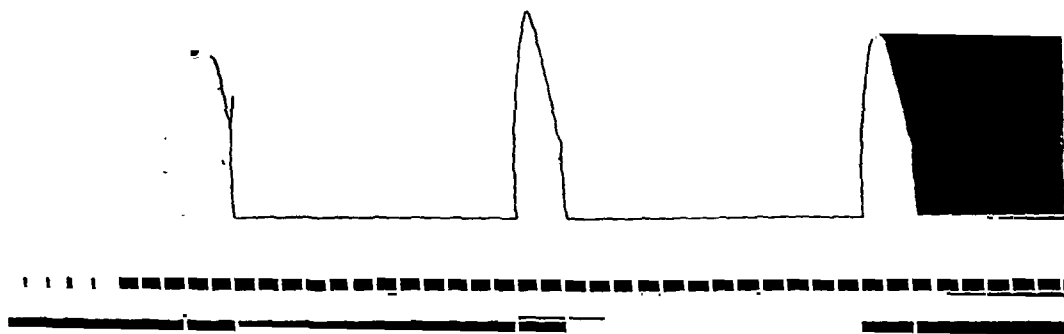


Fig. 5. Muscular responses to currents of constant peak voltage and various rates of exponential rise. The peak voltage was 4 times the rheobase. Time signal: 5 sec. Between the successive pairs of lower signals descending currents were applied with the following time constants for the rise: 0; 1.3; and 2.0 sec.

ment of the responses to direct-current descending pulses of constant intensity. The threshold to the make of the ascending currents was also lowered, as shown by the appearance of responses to that stimulus, absent before hyperventilation.

Not only did acapnia result in an increase of the rapidly accommodating, brief responses which direct currents may elicit, but also in an increase of the long sustained effects which may follow these brief responses (fig. 7). It may be concluded that the threshold for these prolonged responses is also lowered by hyperventilation.

As shown by figures 6A and 7, the changes produced by hyperventilation took place at first rapidly and then more gradually. After 10 to 15 min., how-

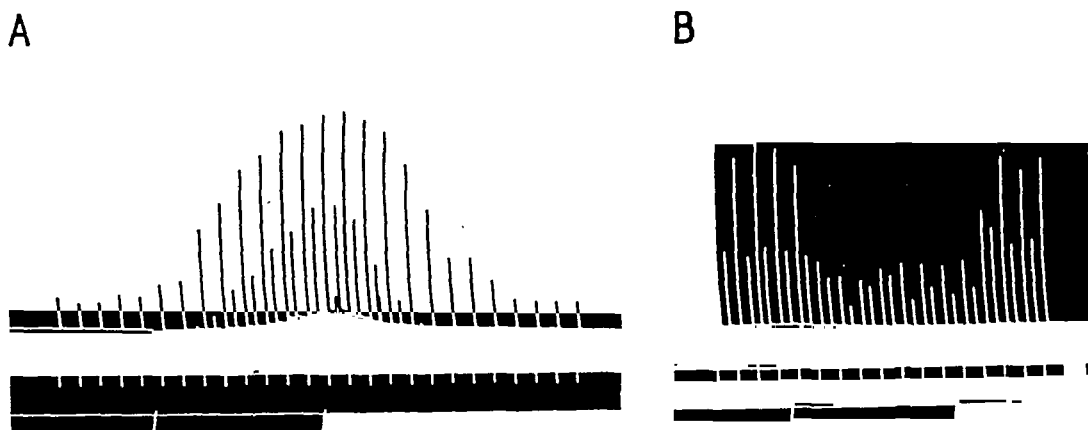


Fig. 6. Effects of hyperventilation and of hypercapnia on the rheobase. Time signal: 30 sec. Direct current pulses of 1 sec. duration were applied at 15-sec. intervals, with alternating polarity.

A. The intensity was the normal rheobase (1.2 v.). The lower signals indicate the beginning and end of a period of hyperventilation. The responses before hyperventilation correspond to the make of the descending currents. These contractions increase during hyperventilation, and in addition responses to the make of the ascending currents appear and increase progressively.

B. Intensity 5 times the normal rheobase. Stimuli applied as in A. Between the lower signals a moderate stream of CO<sub>2</sub> was delivered to the tracheal cannula. The large contractions before CO<sub>2</sub> correspond to the make of the descending currents and the small contractions to the make of the ascending currents. Both responses decreased during the period of hypercapnia, particularly the responses to descending currents.

ever, a steady equilibrium was reached, and it was then possible to make the series of observations necessary for the construction of a peak voltage-time constant curve. Figures 3 and 8 illustrate typical experiments. The whole curve during hyperventilation was lower than the normal curve—i.e., for any time constant of the exponentially rising currents less voltage was necessary for stimulation than in the normal nerve. The change of threshold was especially noticeable for slowly rising currents, those which plot as the second segment of the curves, after the break. The break occurred at a lower time constant than normal (fig. 3A); it sometimes became apparent only during hyperventilation (fig. 8A).

The degree of hyperventilation determined the magnitude of the changes produced. It was thus possible to cause relatively small changes by moderately

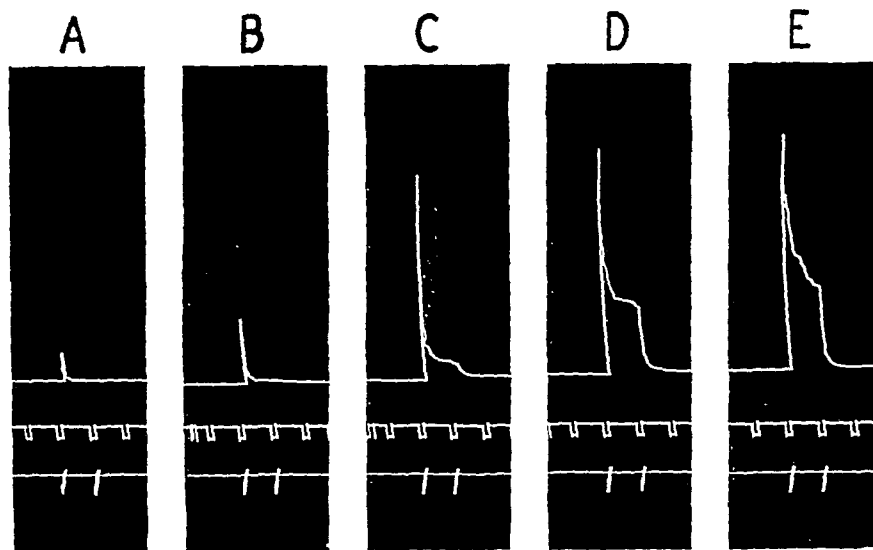


Fig. 7. Increase of the repetitive discharge of nerve in response to direct currents during the course of hyperventilation. Time signal: 2 sec. Descending direct current pulses of rheobasic intensity (1.2 v.) were applied for 2 sec. at 1-min. intervals.

A, normal control.

B to E, 1, 3, 5 and 7 min. after beginning of hyperventilation.

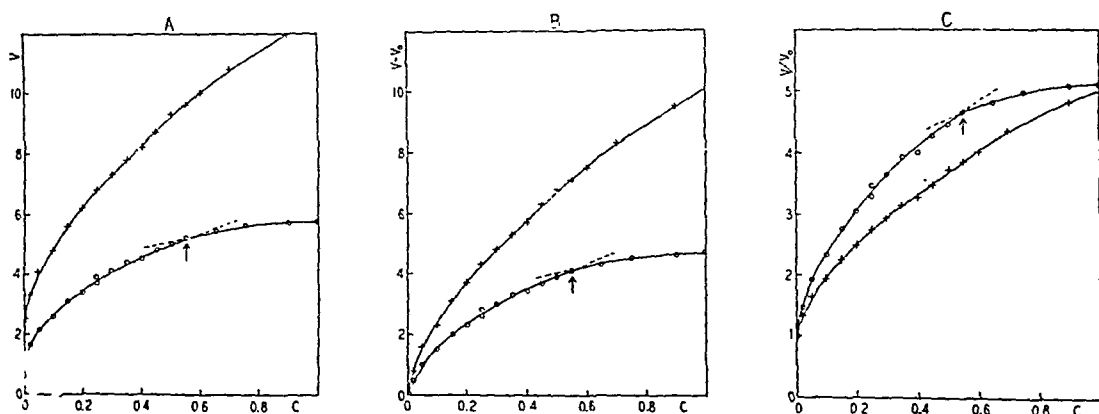


Fig. 8A. Peak voltage-time constant curves before (crosses, table 1d) and during (circles, table 1e) hyperventilation. The arrow has the same meaning as in figure 3.

B. As in A, but ordinates: difference between the peak voltage for a given time constant and the rheobase (time constant 0).

C. As in A, but ordinates: ratio of the peak voltage for a given time constant to the rheobase.

abundant artificial respiration; a later increase of ventilation resulted then in more marked effects.

As illustrated in figure 6A, the changes produced by hyperventilation were not permanent. Removal of the artificial respiration was followed by a return



toward the normal condition. With moderate and not very prolonged hyperventilation a satisfactory reversibility of effects was seen. This is the case in figure 6A. With strong and prolonged artificial respiration hysteresis became apparent. The nerve did not return to its normal characteristics but usually overshoot—i.e., the rheobase and the peak voltage-time constant curve were higher than normal after recovery from a period of increased ventilation.

The sensitivity of the nerves to changes in respiratory rate and depth deserves emphasis. Even very slight hyperventilation resulted in striking changes similar to those illustrated in figures 3, 6A and 8. In some animals a sudden change of the spontaneous respiratory activity caused, for instance, by turning on a heating pad, could produce a significant shift of the peak voltage-time constant curve.

*C. Hypercapnia.* Increase of the  $\text{CO}_2$  tension in the blood caused, like hyperventilation, prompt and marked changes in the nerves. As shown in figures

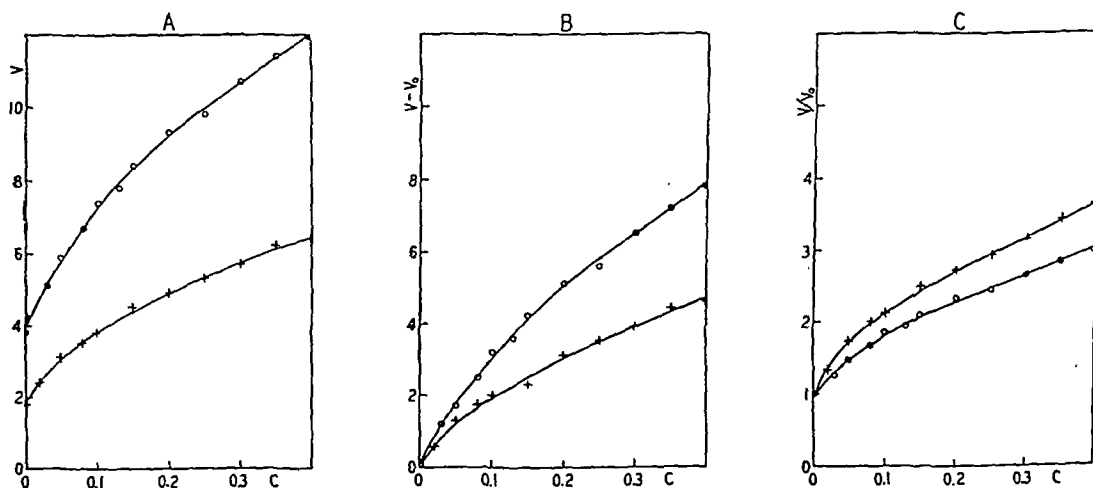


Fig. 9. As in figure 8, but illustrating the effects of hypercapnia. Normal curve: crosses (table 1f). During hypercapnia: circles (table 1g).

6B and 9A, these changes were opposite to those produced by acapnia. With increased  $\text{CO}_2$  the rheobase rose; the peak voltage-time constant curves also rose, particularly the second segment of the curves.

As was the case with hyperventilation, the effects of increased  $\text{CO}_2$  were totally (fig. 6B) or partially reversible. Hysteresis was seen here as an undershooting—i.e., as an incomplete return to normal—instead of the overshooting observed after recovery from hyperventilation.

*D. Veratrine.* The reasons for testing the effects of this drug will be stated in the discussion. The results were similar to those described above for hyperventilation (figs. 3 and 8); they are therefore not illustrated further. The reversibility of these effects was not tested, because the action of the drug is quite long-lasting (several hours) in mammalian nerves.

**DISCUSSION.** I. *The peak voltage-time constant curves.* In this section will be considered only the smooth curves, such as that in figure 2A, and the lower

part of the broken curves, such as that in figure 3A (crosses). The significance of the break is discussed in the next section.

The curves indicate what maximal intensity is necessary in order to stimulate with a given time constant for the rise of an exponential current. This intensity is not necessarily the same as that at which stimulation occurs. As a relatively slowly rising current is passed through a nerve the threshold increases progressively. If at any time the excitatory process developed by the current reaches the threshold, stimulation will ensue. The current, however, may not have reached its peak value at that time. Indeed, it is likely (see Hill, 1936) that with currents of slow exponential rise the current is significantly below its maximal value at the moment of threshold stimulation.

Although the voltages plotted do not correspond directly to the change of threshold, the curves provide an indirect measurement of this change. Thus, if a nerve in certain experimental conditions accommodated more rapidly or to a greater extent than normally, the curve obtained in these conditions would rise more steeply and to a higher level than the normal curve, as the time constants of the currents increase. The detailed interpretation of a given curve, or the comparison of different curves with each other, depends, however, on the definition of accommodation adopted and on the assumptions made for the influence of the intensity of the current on the rate and degree of accommodation.

Hill (1936) assumed that the final degree of accommodation to a current of any fixed intensity is always a constant value above the degree of excitatory process developed—i.e., that after direct current has been applied for a long time the threshold of the nerve to a test shock would be normal at the cathode of the direct current. He further assumed that accommodation is proportional to the degree of excitatory process developed by the current. From these assumptions he derived the following mathematical expression for the peak current-time constant curves:

$$I_2/I_0 = (a/\lambda)^{\frac{a/\lambda}{a/\lambda - 1}} \quad (1)$$

where  $I_2$  is the peak intensity for any given time constant,  $I_0$  is the rheobase,  $a$  is the time constant of the currents, and  $\lambda$  is the time constant of the process of accommodation. This equation is supposed to be applicable for values of  $a$  greater than  $10k$ ;  $k$  is the time constant of excitation of the nerve.

The value of  $k$  for the motor fibers to the Achilles tendon muscles was determined by Rosenblueth and Dempsey (1939) and by Rosenblueth (1940), using Hill's (1936) method, and was found to vary from 0.05 to 1.5 msec. Equation (1) should therefore apply to the present curves from the point corresponding to  $0.2\mu F$  onwards. This applicability was tested lineally as follows. Equation (1) may be rewritten

$$\log (I_2/I_0) - \log (a/\lambda) = \lambda \log (I_2/I_0)/a \quad (2)$$

Since  $I_2$  is proportional to the peak voltages, and  $a$  is proportional to the capacity, it follows from equation (2) that if  $\log (V/V_0) - \log C$  is plotted against  $\log$

$(V/V_0)/C$  (where  $V$  is the peak voltage,  $V_0$  is the rheobase, and  $C$  is the capacity) a straight line should ensue.

Although occasionally a good lineal test developed, the results illustrated in figure 2B (circles) were the rule. Not only did the points deviate from a straight line for the small capacities, to which equation (1) is not supposed to apply, but the points corresponding to relatively high capacities also deviated from a straight line. It may be inferred that Hill's equation does not fit the experimental data, and hence that the assumptions which led to that formula are in turn contrary to experimental facts.

The following equation was found to fit with accuracy all the curves constructed during the experiments:

$$V - V_0 = m a^n \quad (3)$$

where  $V$ ,  $V_0$  and  $a$  have the same meaning as before,  $m$  is a proportionality factor and  $n$  is a fraction ( $1 \geq n > 0$ ). According to this equation  $V - V_0$ , which is indicative of the rise of threshold, is a parabolic function of the time constant of the exponential rise of the currents. Equation (3) may be rewritten

$$\log (V - V_0) = \log m + n \log a \quad (4)$$

Accordingly, if equation (3) is adequate, the plot of  $\log (V - V_0)$  against  $\log C$  should yield a straight line with slope equal to  $n$ . This lineal test is illustrated in figure 2B (crosses). As is the case in this figure, the points corresponding to very small capacities were frequently off the straight line, but their deviation was unsystematic. This deviation is attributed to the difficulty of measuring accurately both  $V_0$  and the  $V$  corresponding to these points. The test is such that slight errors become greatly magnified at that range.

With the exception of the points corresponding to capacities less than  $0.05\mu F$  (time constant less than 2.7 msec.) all the smooth curves yielded excellent lineal tests for a parabola. The first segment of the broken curves also gave a good lineal test (fig. 3B). The curves are therefore fully described if the 3 parameters  $V_0$ ,  $m$  and  $n$  are determined.  $V_0$  is measured experimentally. The other parameters can be directly read from the position of the straight line in the double logarithmic plot;  $n$  is the slope of the line and  $m$  is obtained from the intercept with the  $V - V_0$  axis.

Hill's equation (1) reaches a limiting slope, hence Hill's suggestion that  $\lambda$ , the time constant of accommodation, may be calculated from this slope. The derivative of  $V - V_0$  with respect to  $a$  in equation (3) is

$$d(V - V_0)/da = mn/a^{1-n} \quad (5)$$

This derivative is the slope of the curve and decreases indefinitely as  $a$  increases without limit. In other words, the parabola of equation (3) has no limiting slope. Since all the curves tested fitted equation (3) better than equation (1) it may be inferred that the use of a limiting slope to measure the time constant

of accommodation is not justified by the data. The second derivative of (3) with respect to  $a$  is

$$d^2(V-V_0)/da^2 = mn(n-1)/a^{2-n} \quad (6)$$

which indicates that the slope varies at first rapidly with  $a$  and then progressively more slowly as  $a$  increases. For this reason the curves may suggest a limiting slope, unless analyzed closely.

The average measurements for normal nerves were as follows. The rheobase was usually 3v. potential drop in the voltmeter  $V$  of figure 1, with extreme variations of 5.5 and 1.2v. These values correspond to an average current of 14 $\mu$ amp. (25 to 5.5), and to an average potential drop in the nerve of 55 mv. (100 to 22). The constant  $\lambda$ , measured according to Hill's (1936) method was 18 msec. (40 to 11.5). The constant  $n$  (equation 3) was 0.67 (0.33 to 0.48). There was no correlation between any of the three measurements—i.e., a low rheobase did not correspond consistently to any extreme value of  $\lambda$  or  $n$ , and similarly for the others.

Peak voltage-time constant curves for exponentially rising currents have been studied previously (see Cardot and Laugier, 1913; Schriever, 1930; Delville, 1934; Solandt, 1936a and b; Bernhard, Granit and Skoglund, 1942). In many cases the curves are similar to those illustrated in figure 2A and in the first segment of the curves in figure 3A. All the authors agree that the curves approach straight lines for currents of relatively long time constants. This interpretation is obviously a satisfactory first approximation (see figs. 2A and 3A). The analysis of the curve as a whole, however, shows that a parabolic equation with a fractional exponent gives a better description (figs. 2B, crosses, and 3B).

Curves which were straight lines from the start (including short time constants) were often seen by Cardot and Laugier, Schriever, and Solandt. Equation (3) becomes of course a straight line if  $n$  is equal to 1. In the present observations  $n = 1$  was seen only in one nerve, after prolonged experimental handling—i.e., after several curves had been constructed in which  $n$  was less than 1. Invariably  $n$  was a fraction in fresh nerves. Usually, if an experiment was prolonged, after several procedures had been applied to the nerve, both the rheobase and  $n$  tended to increase. The straight lines ( $n = 1$ ) observed by Cardot and Laugier, Schriever, and Solandt, were probably common in their experience, therefore, because these authors used excised nerves, as opposed to the circulated nerves observed here.

Curves with an initial concavity upwards, such as were reported by Delville, or curves which become a horizontal straight line for large time constants, such as were found by Bernhard, Granit and Skoglund, were never seen in our experiments.

II. *The significance of the break in the peak voltage-time constant curves.* Although, as pointed out above, the curves do not have a fixed slope, their slope at a given point denotes the rate at which the threshold changes when the current is made to rise more slowly—i.e., the slope is an indication of the rate of

accommodation. The break (see fig. 3A) suggests, therefore, a transition from a rapidly accommodating (steeper segment of the curve) to a slowly accommodating process (flatter segment of the curve). The type of response corresponding to each of the two segments agrees with this interpretation. The responses of the first segment are brief (fig. 4A), thus showing rapid accommodation, while those of the second segment are prolonged (fig. 4C), thus indicating slow accommodation.

It may be inferred, therefore, that the nerves have two time constants of accommodation. This inference leads to the further conclusion that the nerve has two thresholds to electric currents. These two thresholds may vary independently. Thus, hyperventilation (figs. 3 and 8),  $\text{CO}_2$  (p. 638), and veratrine (p. 638), all affect the second segment of the peak voltage-time constant curves more than they affect the first segment—i.e., the slowly accommodating threshold is altered more than is the rapidly accommodating threshold. Similarly, in figure 7C to E, the slowly accommodating threshold is seen to decrease at a time during hyperventilation when the rapidly accommodating threshold is changing only slightly.

The existence of two independent thresholds leads in turn to the conclusion that the currents may stimulate nerve by two different independent processes. For rapidly rising currents the threshold of the rapidly accommodating process is lower. This type of response has been more frequently studied and some of the laws which preside in this mode of stimulation are therefore well understood. If a rapidly rising current is delivered which is higher than the two thresholds, a first rapidly accommodating burst of impulses may be followed by long sustained discharges (fig. 5, 1st response). With slowly rising currents, on the other hand, the threshold for the rapid mode of stimulation recedes ahead of the current, while the more slowly accommodating second threshold may be attained and a pure second mode of stimulation may ensue (fig. 4C and D; fig. 5, 3rd response).

III. *The measurement of accommodation. Hypo- and hypercapnia.* As shown in figures 3A, 8A and 9A the peak voltage-time constant curves of nerve are markedly modified by these experimental procedures. The question arises how to qualify these changes with regard to accommodation. Specifically the questions may be asked whether a nerve accommodates more or less than normally during hypo- or during hypercapnia. If the rheobase did not change, those questions would be easy to answer; a direct comparison of the curves would suffice. But since the rheobase is changed the answer is not obvious.

Accommodation has been defined as the increase of threshold during the passage of current. The degree of accommodation will then depend on the degree of change of threshold. Two possibilities are available for the comparison of different curves. The change in threshold may be measured as the difference between the currents necessary to stimulate after and before accommodation. In figures 8B and 9B are plotted these differences ( $V - V_0$ ) against the corresponding time constant (C). The inference from the curves in these figures would be that nerves accommodate less than normally during hyperventilation, and more than normally during hypercapnia.

An alternative measure of the change in threshold is the ratio of the threshold after accommodation to the rheobase ( $V/V_0$ ). In figures 8C and 9C are plotted such ratios against the time constant of the currents. The position of the curves in these figures suggests that nerves accommodate more than normally during hyperventilation, and less than normally during hypercapnia—i.e., a conclusion precisely opposite to that reached when the difference  $V-V_0$ , instead of the ratio  $V/V_0$ , is used as a criterion of accommodation.

To select either of the two criteria,  $V-V_0$  or  $V/V_0$ , as the standard, is to define arbitrarily accommodation in one of the two possible ways. There is no obvious reason for preferring one or the other formulation. The main difference in the two procedures is that when  $V-V_0$  is used the unit of threshold is the volt, or the ampere if the e.m.f. be converted into current, whereas when  $V/V_0$  is employed the unit of threshold is the rheobase. The use of  $V-V_0$  appears, therefore, preferable, because it adopts a standard unit and does not lay undue emphasis on the rheobase—i.e., it does not make the unnecessary assumption that the amplitude of the rheobase is a factor which determines the degree of accommodation.

This discussion of the measurement of accommodation does not follow the lines suggested by Hill (1936). As already mentioned, Hill assumed that after a sufficient time of application of a current of any intensity the threshold of the nerve would always be the same. This assumption is contrary to experimental fact. If the excitability at the cathode of direct current is measured at different times after the application of the current it is found that a level is reached after a brief period. This level is steady for minutes (up to 10 min.) and denotes an increase of excitability above normal, which is approximately proportional to the intensity of the current, within a wide range (unpublished observations).

Since Hill assumed an invariant degree of final accommodation the only measurement he considered of importance was that of the rate of development of accommodation. The data indicate, however, that the degree of accommodation is not constant, but is a function of the intensity of current. For this reason the discussion here has been concerned not only with the time constant of the rate of accommodation, but also with the degree of accommodation to a current of a given final intensity.

Summarizing the effects of hyperventilation on nerve, the following statements may be made. The most obvious change is in the rheobase, which can decrease to one-third the normal value. The constant  $\lambda$ , calculated by Hill's (1936) method, is practically unchanged. The value of  $n$  in equation (3) decreases. A comparison of the changes of threshold produced by slowly rising currents during hyperventilation with the normal changes indicates that hyperventilated nerves accommodate less than normally, if these changes are measured as a difference in the stimulating voltage (fig. 8B). During recovery from hyperventilation the changes were opposite in sign (p. 637). The effects of hypercapnia were also opposite in sign to those seen during hyperventilation (fig. 9).

IV. *Veratrine*. Among other striking effects of veratrine on nerve, characteristically the responses to single shock stimulation become repetitive (Dun and Feng, 1940; Acheson and Rosenbluth, 1941). These repetitive discharges occur long after the stimulus has ceased. They may, therefore, be quite un-

related to the phenomenon of accommodation, in which the interesting feature is the behavior of nerve during the passage of current, not afterwards.

This view differs from that adopted by Cowan and Walter (1937), who state that since nerves treated with tetra-ethyl-ammonium iodide respond repetitively to single shock stimulation, it was to be expected that their accommodation would be slower than normal.

The observations with veratrine emphasize that repetitive responses to single shocks need not be associated with a significant decrease of the rate of accommodation. The main change produced by the drug in the first segment of the peak voltage-time constant curves was to decrease the rheobase (p. 638). The slope of the curves at any given relatively large time constant, on the other hand, was only slightly decreased. The measurements of  $\lambda$  in a typical observation were 12.3 msec. before, and 11.3 msec. after a dose of veratrine sufficient to cause marked repetition.

Apart from the change of rheobase, the part of the curves most affected by veratrine was the second segment (p. 638). Since the change was similar to that produced by hyperventilation (figs. 3A and 8A), and since single shocks did not lead to repetitive responses during hyperventilation, it may be concluded that the repetitive discharges of veratrinized nerves do not depend on the effects of the drug on the second process of excitation and accommodation revealed by the second segment of the curves.

V. *Some properties of nerve suggested by the data.* In addition to the inference that currents may stimulate nerve by two different independent processes (p. 642), the data have a bearing on some aspects of other properties of nerve, as follows.

It has been classical to consider nerves as very resistant to the absence of circulation. Yet it is interesting to note that usually in studies on excised nerves some time is allowed to pass after dissection before the beginning of the study. The statement is frequently made (see e.g., Lucas, 1907) that during approximately the first hour after excision nerves are in an unsteady condition. Lucas (*loc. cit.*) considered two possibilities to explain this instability: the injury done by section and excision might be the responsible damaging factor; or else the nerve may slowly adjust from its normal to a new relatively steady state. The present data emphasize the importance of the circulation of nerve for optimum function. Section of the nerve did not cause instability, but inadequate circulation resulted in a progressive deterioration (p. 631).

The extreme sensitivity of some nerves to electric currents may be stressed. Normal nerve could have a rheobase as low as 1.2 v., measured at the voltmeter *V* in figure 1. During hyperventilation the threshold for direct current could drop to 0.8 v. In terms of current these values indicate thresholds of 5.5 and 3.8  $\mu$  amp., respectively. The drop of potential across the nerves was in turn only 22 to 15 mv., values which are of the order of magnitude of the usual demarcation potential.

The parabolic relationship (equation 3) found between the increment of threshold and the time constant of the exponentially rising currents entails an unexpected corollary. If the curves should remain parabolic beyond the range

explored, any current, no matter how slow its rise, would be capable of stimulating if the intensity were increased sufficiently. This corollary is in contradiction with the generally accepted notion (Lucas, 1907) that there is a limiting rate of rise of lineally increasing currents below which no stimulation will ensue no matter how strong the current becomes.

Admittedly the inference reached here, that a slowly rising current would always stimulate if it were made sufficiently intense, is an extrapolation of the curves in figures 2A, 3A, 8A and 9A. Practically a very slowly increasing current would destroy the nerve before stimulation. It should be noted, however, that Lucas' conclusion of a limiting slope of lineal increase is also an extrapolation to infinite. What Lucas observed experimentally was that currents with a slow gradient did not stimulate even when they ultimately reached a voltage 8 times the rheobase. In the present observations it was always possible to stimulate with currents of peak voltage of 6 to 10 rheobases, even when their time constant of rise was 4 sec., i.e., when after 4 sec. they had only reached an intensity of approximately 4 to 7 rheobases (fig. 4D).

#### SUMMARY

Accommodation, that is, a change of threshold during the passage of current, was studied in circulated cat's nerves by measuring the peak voltage for threshold stimulation by an exponentially rising current of variable time constant (fig. 1).

The peak voltage-time constant curves were smooth (fig. 2) or they had a break (fig. 3), thus indicating the existence of two segments. The responses plotted in the first segment differed from those plotted in the second (fig. 4). Both types of response, brief and protracted, could be seen with suprathreshold voltages and rapidly rising currents (fig. 5).

Hyperventilation and veratrine caused a decrease of rheobase (fig. 6A) and a lowering of the curves (figs. 3 and 8, p. 638); hypercapnia resulted in a rise of the curves (fig. 9).

The curves do not fit the theoretical equation which was suggested by Hill (1936; equations 1 and 2; figs. 2B and 3C). They are adequately described by a parabolic relationship between the increment of voltage and the corresponding time constant (equations 3 and 4; figs. 2B and 3B). This relationship implies that there is no limiting slope (equation 5).

The break in the curves denotes two different thresholds with different time constants of accommodation (p. 642). From these two thresholds it is inferred that currents may stimulate nerve by two different independent processes (p. 642).

The measurement of accommodation is discussed (p. 642). The existence of a critical lineal gradient for stimulation by direct current is rendered unlikely by the data (p. 644).

We are indebted to Dr. Norbert Wiener of the Massachusetts Institute of Technology for stimulating advice and criticism.



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## SOME ACTIONS OF INDOLE ON THE DOG

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Ever since the work of Herter (1), the effects of indole in the body have been discussed. The origin of indole is usually considered as intestinal, but Doland (2) mentions that morbid conditions of the mouth and throat as well as gangrene and abscess of the lung favor the production of indican. Some investigations have been made on indole in cold-blooded animals (3, 4), and also on isolated tissue of warm-blooded animals (5), but it is the purpose of the present paper to study the effect of indole on anesthetized dogs.

**METHODS.** Dogs were anesthetized with nembutal and prepared for recording arterial blood pressure, respiration and intestinal movements. The blood pressure was obtained from the carotid artery, respiration by means of a pneumograph about the thorax, and the intestinal movements by means of a balloon in the duodenum. The indole was dissolved in propylene glycol or in olive oil, all other drugs were dissolved in water. Injections were made into the femoral vein unless otherwise stated.

**RESULTS.** Twenty-two dogs were used. Figures 1A and 1B illustrate the typical effects obtained. Doses of 25 mgm. of indole per kilo produced rapid effects which gradually diminished after about 15 minutes. There is a momentary apnea followed by a marked increase in the rate and usually the depth of respiration. The blood pressure falls abruptly and then slowly and gradually returns to normal. Intestinal tone and movements varied: usually there was a fall of tone and movement, occasionally there was a rise in the tone with increased movements, and in a few cases there were mixed effects. Salivation was usually increased and mucous secretions accumulated about the nostrils. Spasms of the paws and jaws usually developed and frequently generalized clonic convulsions appeared, gradually disappearing after about 10 to 15 minutes.

Various methods of administration were tried and it was found that the usual doses given subcutaneously, intermuscularly, or orally were without effect. Intraperitoneal injections produced the same reaction as the intravenous injections.

Atropine in doses of 1 mgm. per kilo had no effect on the indole reactions on the blood pressure, respiration, intestines and convulsions, but it did seem to decrease salivary and mucous secretions.

Doses of 60 mgm. of indole per kilo injected intravenously caused intensification of all the above symptoms without a return towards normal. Death resulted from the continuous fall of blood pressure to zero. The autopsy of animals killed with indole showed the following: the lungs were edematous and

bloody mucous secretions were seen in the trachea, the abdominal organs showed a marked passive congestion and the heart was usually dilated.

**DISCUSSION.** Yanai (4) has reported that indole will produce convulsions when injected into the frog. In the present work indole regularly produced convulsions when given intravenously or intraperitoneally.

Ott and Ulman (3) and Danilewski (6) report that indole lowers the force and the frequency of the frog's heart. Waddell (5) who studied the action of indole on excised warm and cold-blooded animals' hearts also noted a diminished ampli-

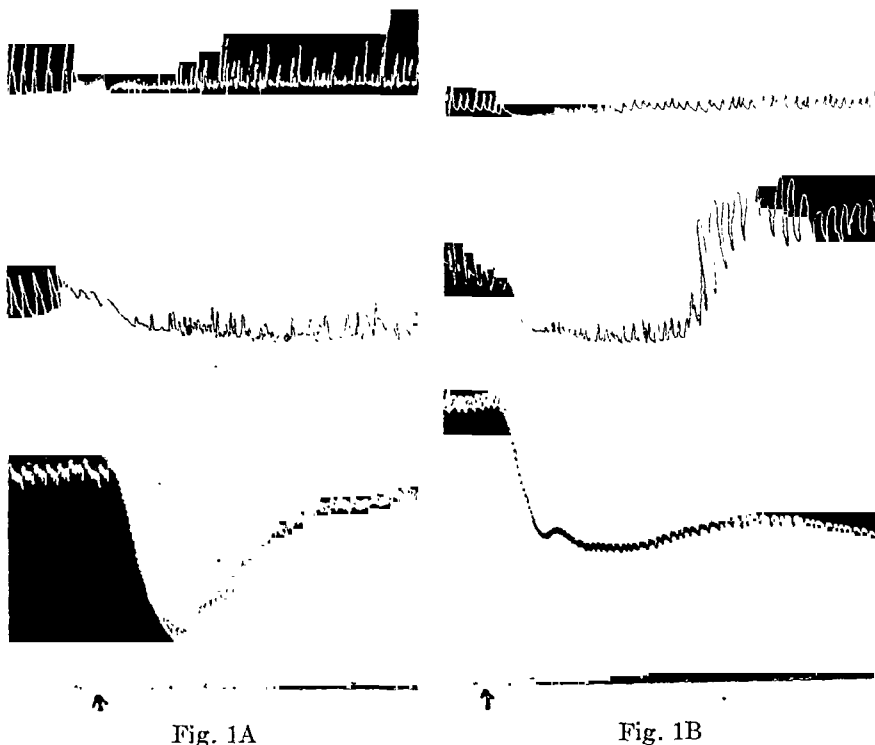


Fig. 1A

Fig. 1B

Fig. 1. Upper tracing, respiratory record; middle tracing, intestinal movements; bottom tracing, blood pressure record. Figure 1A, 25 mgm. of indole per kilo of body weight were injected at arrow; note apnoea and convulsive movements with fall of intestinal tone and blood pressure. Figure 1B, 60 mgm. of indole per kilo of body weight were injected at arrow; note apnea, rise of intestinal tone, and fall of blood pressure.

tude and output. Biebl (7) reports a congestion of the liver and other organs from the action of indole. The above reports may explain the fall of blood pressure that was always observed in this work.

The salivation and mucous secretions reported here on the dog may be inferred from the work of Biebl who found that chronic effects of indole produced a pneumonia of the broncho-lobar type. Guggenheim and Loeffler (8) studied the action of indole on intestinal strips of guinea pigs and found that there was first a rise and then a fall of the tone. This also agrees with many of our experiments.

Waddell *loc. cit.* from his work believes that indole acts so as to depress the

heart muscle directly since atropine and indole seem to depress smooth muscle in general. We also found that atropine did not interfere with many of the actions of indole.

The duration of the effects is probably due to the fact that indole remains in the blood stream only a short time, as has been reported by Houssay.

#### SUMMARY

In dogs anesthetized with nembutal, indole injected intravenously in doses of 25 mgm. per kilo produces:

1. A short period of apnoea followed by an increase in respiration.
2. An immediate fall of systemic blood pressure.
3. Usually a rise in the intestinal tone followed by a fall.
4. Increase of mucous and salivary secretions.
5. Chronic convulsive movements (which with larger doses become generalized).
6. The effects are not abolished by atropine.

Death which occurs with large doses of indole is usually due to dilatation and arrest of the heart action.

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# THE ETIOLOGY OF HYPERICISM, A PHOTSENSITIVITY PRODUCED BY ST. JOHNSWORT

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Light sensitivity has latterly become regarded as a discrete clinical entity. However, experimental studies of this general syndrome are rather scarce. In his recent monograph Blum (1) has presented an extensive and critical review of the entire field, and one of the most clear-cut photosensitivities he discusses is the disease of domestic animals known as hypericism. The disease has long been recognized as due to the grazing of domestic animals on the common weed St. Johnswort, *Hypericum perforatum*, with subsequent exposure to sunlight. Blum (1) points out the lack of certain evidence to establish the etiology of the disease. An inquiry into this problem seemed to promise not only a better understanding of hypericism, but a contribution to the general knowledge of photosensitization.

Hypericin, a red fluorescent pigment occurring in St. Johnswort, has been suspected as the causal agent owing to its activity as a photodynamic sensitizer. The pigment was recently studied chemically by Pace and Mackinney (2) and found to consist of several closely related chemical fractions. These fractions are believed to be partially hydrogenated polyhydroxy derivatives of heli-anthrone, which is related to anthraquinone.

The present study is an attempt to establish more exactly the relation of hypericin to the disease, and several lines of investigation have been pursued. First, the presence of hypericin in the skin of laboratory animals previously fed the dried plant was ascertained, and second, the ability of light of various wave lengths to produce characteristic symptoms of hypericism was studied in order to determine the approximate action spectrum. The latter should correspond to the spectral regions of maximal light absorption by hypericin if this pigment is the causal agent (1). In addition to these experiments the effect of oral feeding of purified hypericin was studied.

**EXPERIMENTAL.** *Demonstration of hypericin in the skin.* White rabbits were sensitized by feeding on dried *Hypericum perforatum* with no other food for at least one week. The sensitivity to light was tested by irradiating a small spot one square centimeter in area on the previously depilated flank of the animal by means of the apparatus shown schematically in figure 1. Five to ten minutes' irradiation in this apparatus with only filter  $F_2$  in the optical path was sufficient to evoke a response in sensitized animals. The response, which took the form of a wheal surrounded by erythema and accompanied by symptoms of localized pruritis, was best observed on the day following irradiation. At this time an

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estimate of the degree of response could be made. After several days, a necrosed area occupying the site of irradiation was noted.

Three rabbits whose photosensitivity was tested in this fashion were killed and their skins, livers, and a portion of the skeletal muscle were ground up separately and extracted with 90 per cent acetone in water. These extracts were filtered and examined spectroscopically. The absorption spectrum of hypericin was readily recognizable in all three extracts. Hypericin in this

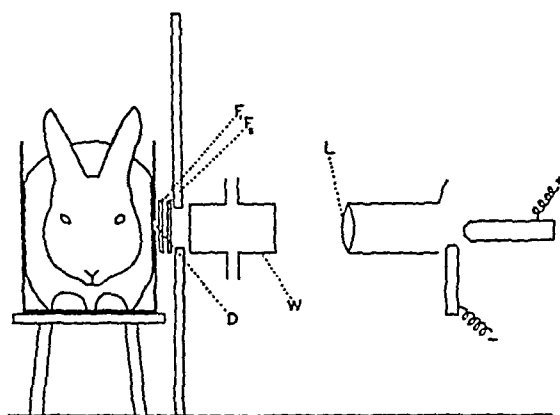


Fig. 1

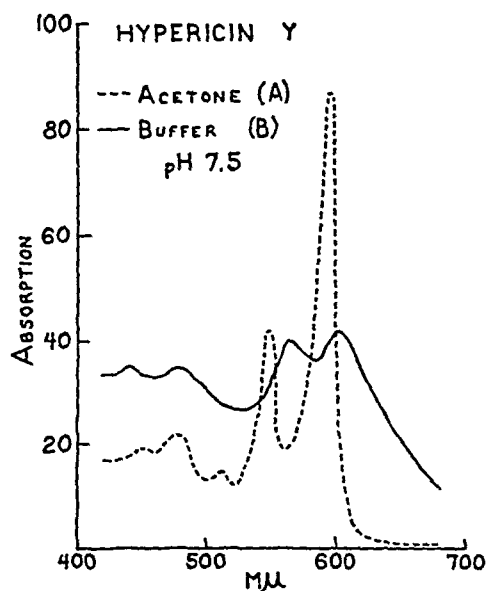


Fig. 2

Fig. 1. Apparatus for determination of action spectrum. The light source is a Leitz-Wetzlar D.C. projection arc using Eveready "Sunshine" carbons. A potential of 50 volts was maintained across the arc. The light was focused on the bare skin of the rabbit by means of the lens, *L*, and the diaphragm, *D*. The circular opening in the diaphragm 1 cm. in diameter served to sharply delimit the area irradiated. Permanently interposed in the optical pathway were two filters, *W* and *F*<sub>1</sub>. *W* is a water filter 8.5 cm. in length which removed most of the infra-red. *F*<sub>1</sub> represents a Wratten gelatin filter (2-A) which is designed to cut off radiation of wave lengths below 400 *mμ*. The third filter, *F*<sub>2</sub>, represents the series of Wratten gelatin filters used to isolate various portions of the spectrum.

Fig. 2. The ordinate is in relative units and does not indicate extinction coefficients of either solution. The figure is intended to show the qualitative difference between the two spectra.

solvent (curve A in fig. 2) displays an intense absorption band at 595 *mμ* with a weaker band at 550 *mμ*. Both bands could be detected in the extracts.

When reflected light from the skin of living sensitized rabbits was examined with a small direct-vision spectroscope, a faint absorption band could be discerned in the vicinity of 605 *mμ*. This corresponds approximately with the main absorption band of hypericin either in acetone or in aqueous solution buffered at pH 7.5. The absorption spectrum of hypericin in this medium is shown by curve B in figure 2. In addition to the faint band at 605 *mμ* the strong oxyhemo-

globin bands at  $540\text{ m}\mu$  and  $577\text{ m}\mu$  were observed. Control animals examined in the same way did not show the additional band at  $605\text{ m}\mu$  nor did aqueous acetone extracts of normal tissues exhibit absorption bands at  $595\text{ m}\mu$  and  $550\text{ m}\mu$ .

These observations seem to establish definitely the presence of hypericin in the skin of animals fed on St. Johnswort and shown to be photosensitive.

*Action spectrum of hypericism.* The ideal manner for study of the action of various wave lengths of light on a photobiological process entails the use of monochromatic radiation to produce the response. However, since the intensities of light required to evoke a response in photosensitive animals are relatively high, this method is impractical. Consequently in the present work recourse

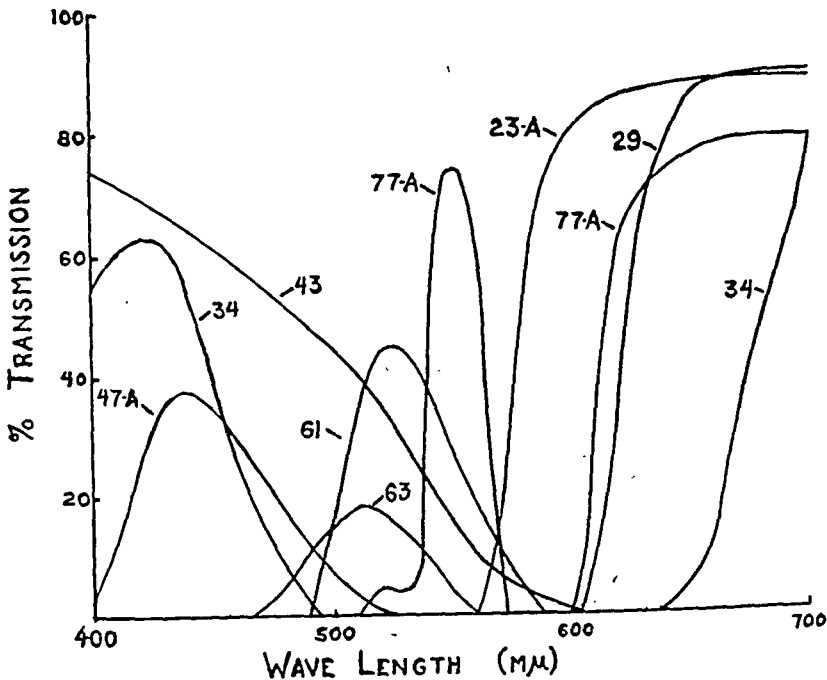


Fig. 3. Curves showing the per cent of light transmitted at various wave lengths by the filters used in this work. The numbers are the Wratten designation for the filters shown.

was had to the use of filters which strike a balance between maximal spectral purity and maximal energy transmission. A series of Wratten gelatin filters was chosen to cover the region of the spectrum from  $400\text{ m}\mu$  to  $700\text{ m}\mu$ . The per cent transmission curves for these filters are shown in figure 3.

Rabbits which had been previously sensitized by feeding on St. Johnswort were irradiated in the apparatus shown in figure 1. This was so arranged that the various filters in figure 3 could be interchanged at position  $F_2$  in the apparatus. Tests were made with each filter on several rabbits. An estimate of the degree of response was made subjectively as described in the foregoing section, and results of the tests are given in table 1.

It was impractical to make more than three determinations on one rabbit because of the long exposures required. For this reason the first exposure was

always made through Wratten filter 23-A to check the animal's sensitivity since the response through this filter after twenty minutes of irradiation was indistinguishable from the maximal response with no filter in the optical path. Thirty minutes of irradiation was the standard period of exposure in all experiments. This period of exposure had no effect whatever on normal rabbits.

TABLE 1

*Results obtained by irradiation of sensitized rabbits through various filters*

The degree of erythema is indicated as follows: +++ strong; ++ medium; + barely perceptible; and 0 no effect. See table 2 for the wave-lengths transmitted by these filters.

RABBIT NO.	WRATTEN GELATIN FILTERS							
	23-A	29	34	43	47-A	61	63	77-A
1	++							
2	+++	0						
3	++		0					+
4	+++						0	++
5	+	0			0			
6	+++				0	0		
7	+++	0		++				
8	+++							++
9	+++		0				0	
10	++		0	+				
11	+++			++		0		
12	+++							

TABLE 2

Column 2 summarizes the results from table 1 for each filter used. Column 3 indicates the predicted ability of each filter to produce a response if hypericin is the photosensitizer. The limits of transmission of each filter are given in column 4. For further explanation see text

(1) FILTER	(2) AVERAGE RESPONSE	(3) AREA UNDER CURVE	(4) REMARKS
23-A	+++	13.44	Transmits from 570 m $\mu$ to red end
29	0	5.99	Transmits from 610 m $\mu$ to red end
34	0	4.70	Cuts out 470 m $\mu$ to 660 m $\mu$
43	++	9.96	Cuts out 560 m $\mu$ to red end
47-A	0	2.94	Transmits from 400 m $\mu$ to 500 m $\mu$
61	0	3.86	Transmits from 500 m $\mu$ to 570 m $\mu$
63	0	1.42	Transmits from 490 m $\mu$ to 540 m $\mu$
77-A	++	10.06	Transmits 540-560 m $\mu$ and 610 m $\mu$ on

A sensitized rabbit was also exposed to sunlight through filters 23-A and 29 alone. As in the case of irradiation by means of the apparatus in figure 1, filter 23-A allowed sunlight to evoke the symptoms of photosensitivity whereas filter 29 protected the animal. Since filter 29 allows almost all the infra-red to pass, this spectral region cannot be capable of causing hypericium.

Table 2 summarizes the results obtained from the experiments with carbon



arc irradiation, and includes a short description of the characteristics of the filters used. Also given in the third column of table 2 are figures which express the predicted effectiveness of the light transmitted through each of the filters if hypericin were the photosensitizer. These values are only gross approximations. They were derived by obtaining for various wave lengths the product of the emission of the light source, the transmission of the given filter in the optical path, and the absorption coefficient of an aqueous hypericin solution buffered at pH 7.5. The area under the resultant curve was then measured. This calculation is described in detail by Blum (1). The energy distribution values for the light source were taken from the National Carbon Company Circular no. CP-1103, *Radiation characteristics of everready sunshine and therapeutic carbons*. These can only be regarded as approximate values. The absorption spectrum of hypericin in the skin was assumed for the purpose of calculation to be that of hypericin in an aqueous buffered solution shown by curve B in figure 2. Although there are numerous inherent inaccuracies in this calculation, the predicted effectiveness of the various wave length regions agrees reasonably well with the observed effectiveness.

The three filters which pass light capable of producing a response, namely, 23-A, 43, and 77-A, have a predicted effectiveness of almost double the other filters used. These three filters transmit the region of the spectrum from 540  $m\mu$  to 610  $m\mu$ , hence the symptoms of experimental hypericism must be produced by visible light of this spectral region. The region of the spectrum from 400  $m\mu$  to 540  $m\mu$  exerts some effect on sensitized animals but not nearly to the same extent as the region 540  $m\mu$  to 610  $m\mu$ . The longer wave lengths beyond 610  $m\mu$  are apparently ineffectual in the production of symptoms of hypericism.

In all these experiments the ultra-violet was eliminated by filter 2-A since facilities were not available for a satisfactory study of this spectral region.

The wave length region 540  $m\mu$  to 610  $m\mu$  agrees fairly well with the region of maximum absorption of hypericin both in acetone and in aqueous solution buffered at pH 7.5 (fig. 2). Pace and Mackinney (3) have described the relatively wide variations in position and intensity of the absorption maxima of hypericin with changes of solvent. Hence, at present any more quantitative comparison of the action spectrum of hypericism with the absorption spectrum of hypericin in the skin awaits a better appraisal of the latter. From the data at hand it may be said that the action spectrum of experimental hypericism agrees approximately with the best available absorption spectrum of hypericin.

*Oral feeding of hypericin.* Pulverized hypericin was rubbed up with 0.5 per cent gum tragacanth solution in water, and the resulting suspension was diluted to contain 20 mgm. of hypericin per cc. The mixture was administered to two 180 grams white rats by stomach tube in divided doses over a period of six hours. One rat received 0.5 cc. every three hours, and the other received 1.0 cc. every three hours. Thus, the rats received a total of 30 mgm. and 60 mgm. respectively of purified hypericin. On the following day these two rats, together with two control animals, were exposed to sunlight through a window glass filter.

In five minutes a distinct erythema of the ears was noted in both of the

hypericin fed rats, and after ten minutes both animals were scratching vigorously and seeking shade. The control rats showed none of these typical symptoms of photosensitivity.

Thus, oral administration of hypericin can cause photosensitization in white rats with a dosage (not minimal) of 167 mgm. of hypericin per kilo of body weight.

DISCUSSION. There has been little doubt that hypericism was due to a combination of the ingestion of *Hypericum* sp. and subsequent exposure to light. It had also been shown that a pigment which can cause photosensitization when injected, i.e., hypericin, could be isolated from St. Johnswort. However, as has been repeatedly pointed out (1), (4), the mere isolation from a plant of a pigment which can cause photosensitization when injected is not conclusive evidence that the pigment is responsible for the natural occurrence of a photodynamic disease. Chlorophyll, for example, may be mentioned as a pigment which can cause photosensitization when injected but which has no effect when eaten. Furthermore, in the disease Geeldikkop certain plants cause photosensitization although they do not contain the responsible photodynamic pigment (1), (5). However, this type of photosensitivity is accompanied by extensive liver damage, and the livers of animals suffering from hypericism are normal.

Thus, before hypericin could be definitely established as the causal agent in hypericism it was necessary to demonstrate its presence in the skin of animals sensitized by feeding on the plant. It was necessary to show that oral administration of hypericin, when isolated from the plant, could produce photosensitization in order to demonstrate the effectiveness of the suspected pigment through the normal port of entry into the body. Finally, a determination of the action spectrum of the disease was undertaken in order to further define the photochemical receptor in the disease.

From the results of these experiments it may be stated that hypericism is caused directly by the ingestion of hypericin. The pigment is apparently absorbed unchanged and distributed throughout the body of the animal. The absorption of light by this pigment brings about destructive changes in skin irradiated with the wave lengths absorbed by the pigment. These changes produce the typical syndrome of the disease.

Blum and Pace (unpublished data) have shown that molecular oxygen is necessary for photodynamic hemolysis using hypericin as the sensitizer. All this evidence indicates that the sensitization to light in hypericism is photodynamic action, i.e., photo-oxidation of readily oxidizable substances in the skin (1).

#### SUMMARY

1. Hypericin, a red fluorescent pigment, has been identified spectroscopically in acetone extracts of various tissues from rabbits sensitized by feeding on *Hypericum perforatum*. Hypericin has also been demonstrated spectroscopically in the skin of a living rabbit sensitized in like fashion.

2. Administration of hypericin by mouth has been shown to cause typical photosensitization in white rats.

3. The region of the visible spectrum responsible for experimental hypericism has been found to correspond, within reasonable limits, to the principal absorption bands of the pigment hypericin in the visible region of the spectrum.

4. It is concluded that hypericin is the photosensitizer responsible for the production of the syndrome of hypericism in domestic animals.

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# WATER METABOLISM OF THE CHICKEN (*GALLUS DOMESTICUS*) WITH SPECIAL REFERENCE TO THE RÔLE OF THE CLOACA<sup>1</sup>

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The majority of workers have held that absorption of water from the cloaca is an essential part of avian economy<sup>(8, 12, 15)</sup>, but the recent work of Hester, Mann and one of us (Essex) casts doubt on the validity of such a conclusion. A review of the literature makes it apparent that the conclusions of the earlier workers on the problem of absorption from the cloaca were drawn largely from qualitative and, for the most part, incidental observations.

The concept of absorption of water from the cloaca appears to have originated with Wiener and was amplified further by Milroy and later by Sharpe<sup>(12, 13)</sup>. The hypothesis was formulated from the observation that birds possessing an artificial anus drank more water than controls. Unfortunately, exact figures were not given by any of these workers, and later studies have shown that the daily volume of urine may be surprisingly small.

In the present paper we have recorded data on the total water balance of the chicken. Studies were made first on birds that had artificial anuses (procedure of Milroy) in order to repeat and extend the observations of previous workers. Following this type of surgical procedure the bird retains the possibility of some reabsorption, as the urine bathes the mucosa of the proctodeum before expulsion. Therefore, a technic was developed by which the ureters were brought directly to the outside. Following this type of surgical procedure, there is no mucosa left, other than that lining the ureters themselves, as possible surface for absorption.

Birds were brought into the laboratory and during control periods the daily consumption of food and water was determined. Subsequently, an artificial anus was created in some birds, while the ureters of others were exteriorized. After an adequate period of recovery, the consumption of food and water was studied again. The urine and feces were collected in separate receiving vessels and examined for amount and for content of water, uric acid and chloride.

**METHODS.** *Construction of the artificial anus.* Figure 1a shows the normal rectum and cloaca of the fowl. With the bird under pentobarbital sodium anesthesia it was secured on its back and a midline incision about 5 cm. long was made, starting about 16 mm. from the anal orifice. The rectum was severed at the desired point and the distal end closed at its junction with the cloaca.

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The rectum was brought through the body wall and sutured to the peritoneum with interrupted silk sutures. The skin was stitched to the peritoneum and drawn inward toward the new external opening of the rectum (fig. 1b and c).

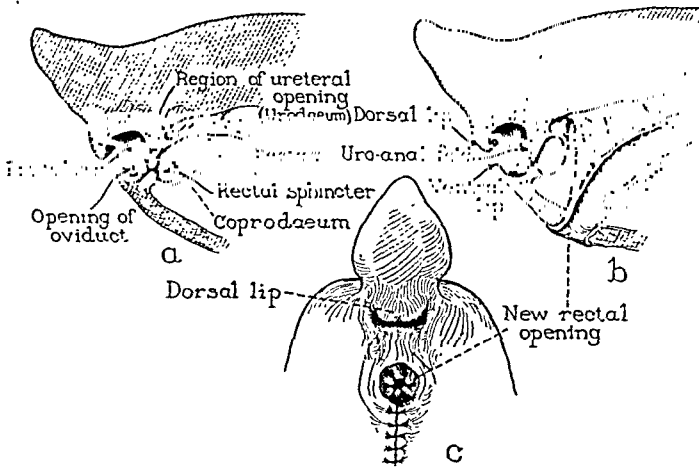


Fig. 1a. Sagittal section of the rectum and cloaca of the fowl; b and c, technic of construction of an artificial anus.

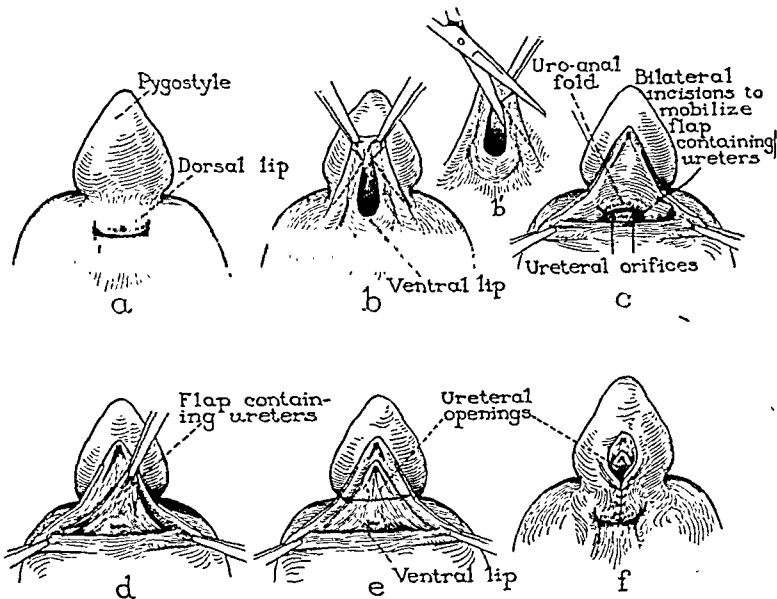


Fig. 2. The technic of exteriorization of the ureters of the fowl. See text for explanation of figure.

*Procedure for exteriorization of the ureters.* Figure 2a shows the region of the cloaca before operation. With the bird under pentobarbital sodium anesthesia the dorsal lip of the cloaca was pulled up by means of forceps as shown in figure 2b. Two cuts were made with the scissors on either side, directed inward and downward (fig. 2b and b'). The result of these two cuts was a triangular flap removed from the dorsal lip. This flap was brought high onto the pygostyle and

fixed to the skin by a silk suture (fig. 2c). The uro-anal fold was brought into view in this way. This fold (fig. 1) separates the urodeum from the proctodeum. The next step of the procedure, and perhaps the most difficult, was to free this fold with the ureters attached and bring it up onto the pygostyle. A cut was made on each side (fig. 2c) and the fold freed from the rectum below and the tissue above by blunt dissection. The flow of urine made it possible to locate the ureters and avoid cutting them. The freed fold was elevated (fig. 2d) and stitched to the triangular flap on the pygostyle (fig. 2e). The dorsal lip of the cloaca then was reconstituted by approximating the lateral portions remaining after removal of the triangular flap (fig. 2f). This closure was made with care, because some trouble was had with sloughing during the healing process.

A discussion of the anatomy of the cloaca which, in the interests of conservation of space we have omitted, may be found in the writings of Boyden (3, 4).

*Procedure for collection of the urine.* By means of a cannula of original design, urine was obtained from birds that had not undergone surgical procedures on the cloaca. This instrument consists of a 10 mm. glass tube about 9 cm. in length. Fused to one end of the tube is a glass ball about 23 mm. in diameter. A large hole is made in the glass tube very close to the ball; its edges are fire-polished and a small flange is turned outward. In the collection process, the ball is inserted into the cloaca and beyond the sphincter of the rectum. The hole in the tube then rests immediately beneath the urodeum. By claspings the ball the rectal sphincter holds the tube in place during the collection period.

Gauze-collodion cannulas were made for the collection of urine from the birds after operation. The cannulas were approximately 20 mm. in diameter and 30 mm. long and they were reinforced with a wire ring at the distal end. A toy balloon was slipped over this ring during the collection process. The proximal end of the cannula was sewed to the skin around the ureteral fistula by interrupted silk sutures. A continuous thread was used to draw the skin more closely about the cannula. In this manner a watertight connection was formed between the cannula and the skin. A harness of cloth was designed to hold the weight of the urine bag and to hold a square of oiled silk for the collection of feces. The birds were free to move about their separate cages in a normal way throughout the collection period. To accustom the birds to the use of the harness they were required to wear it for several days in advance of the collection period.

The estimation of uric acid was done by means of the colorimetric method of Benedict and Franke.

Determinations of the concentration of chloride in the plasma were done on 1 milliliter of plasma, using the modified Volhard-Harvey titration<sup>(11)</sup>; the same method was used for the urine. Determination of the pH of the urine was done with a quinhydrone electrode.

*Physiologic Effects Produced by the Artificial Anus.* None of the birds that had artificial anuses remained suitable for experimentation for more than about three weeks. After about three weeks the gut became atonic and even though the new anus was adequately patent, elimination was slow and obstruction in-

evitable. In spite of these difficulties, very satisfactory experimental birds were obtained and kept in good condition for two or three weeks. One hen (table 1) was kept on a continuous collection period of nine days. Its state of health was excellent during the progress of the experiment but a rectal obstruction developed about a week after this period ended.

*Physiologic Effects of Exteriorization of the Ureters.* Hester and associates reported a procedure for exteriorization of the ureters in which the dorsal portion of the anal ring was elevated onto the pygostyle. This type of operation has the disadvantage that the urine can run back into the coprodeum. In the method presented in this paper the urine is voided directly to the outside at all times without making contact with any appreciable mucosal surface.

TABLE 1

*Water balance study on a hen (bird 1) that had an artificial anus; gut severed just proximal to proctodeum; 1 per cent salt in diet*

DATE, 1940	BODY WEIGHT	FOOD INTAKE	WATER INTAKE	WET WEIGHT FECES	DRY WEIGHT FECES	WATER IN FECES	URINE VOLUME	pH OF URINE	URIC ACID IN URINE
	gm.	gm.	cc.	gm.	gm.	gm.	cc.		gm.
8-21	2,328	38	240	37	8	29	110	6.17	1.65
8-22	2,309	59	265	35	8	27	173	6.75	1.43
8-23	2,310	70	270	39	10	29	144	6.64	1.50
8-24	2,357	89	240	44	10	34	129	6.89	1.46
8-25	2,375	85	195	44	10	34	133	6.83	1.77
8-26	2,366	98	215	63	22	41	127	6.34	
8-27	2,343		250	57	16	41	103	6.34	
8-28	2,428	114	235	76	18	58	123		
8-29	2,404	143	270	74	18	56	115		
Averages.....	2,358	87	242	52	13	39	129		

After the ingestion of large amounts of water (200 cc.) the urine was observed to be watery with shreds of mucus and occasional blobs of uric acid. Birds ingesting smaller amounts of water excreted a less copious urine which often contained a high concentration of solid uric acid. In most birds the urine collected about the fistula as a gelatinous mass of uric acid. The feces became dry and assumed a form quite different from the usual.

If a bird was placed on the standard diet without added sodium chloride, a loss of body weight was observed in the first few days after the surgical procedure (fig. 3). After a variable length of time the hematocrit value was greater than normal and the concentration of chloride in the plasma was less than normal. If sodium chloride was not added to the diet, the bird continued to lose body weight until it died. Extreme loss of body water was apparent in the almost total loss of muscle volume, the deep sunken eyes and the black, shriveled comb. At this point concentrations of chloride were found as low as 328 mgm. per 100 cc. of plasma (normal 390 to 425 mgm. per 100 cc.) and hematocrit values as high as 60 per cent (normal 30 to 40 per cent).

If sodium chloride was added to the diet in any manner, the foregoing changes were prevented or corrected. One per cent salt in the food was sufficient to accomplish this result. In many cases drinking water containing 0.2 per cent sodium chloride was placed in the cage and the bird permitted to make its own choice between tap water and salt water. Intraperitoneal injections of Ringer's solution or physiologic saline solution also were used. When salt was given to the bird in any of these ways, a rapid and prompt response was obtained. The body weight increased rapidly; the hematocrit and plasma chloride values re-

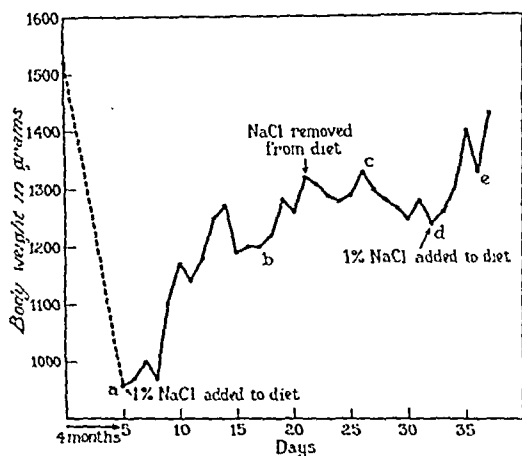


Fig. 3

Fig. 3. Restoration of normal body weight by the addition of 1 per cent salt to the food of a chicken (bird 9). *a*, Concentration of chloride 328 mgm. per 100 cc. of plasma, hematocrit value 54 per cent; *b*, concentration of chloride 416 mgm. per 100 cc. of plasma, hematocrit value 32 per cent; *c*, concentration of chloride 412 mgm. per 100 cc. of plasma, hematocrit value 32 per cent; *d*, concentration of chloride 410 mgm. per 100 cc., hematocrit value 43 per cent; *e*, hematocrit value 38 per cent.

Fig. 4. Loss of body weight when drinking water was withheld from control chickens and chickens having exteriorized ureters.

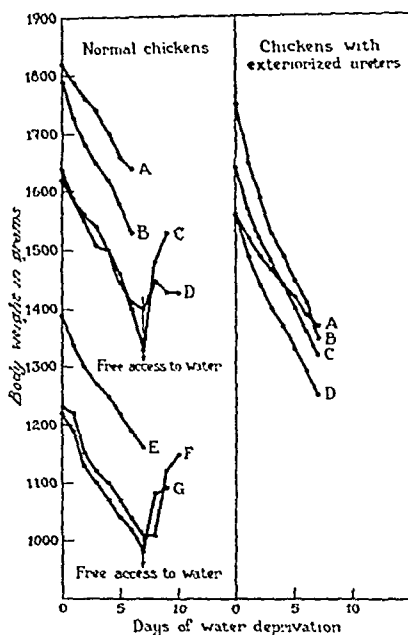


Fig. 4

turned to normal almost immediately. If salt was withheld again, loss of weight occurred as before.

Bird 9 furnished the most complete data we obtained on these effects. After exteriorization of the ureters this hen was placed on a diet without added salt. The wound healed completely in five days but loss of weight was rapid and progressive. After six weeks the weight of this hen had decreased from the initial value of 1,300 grams to 800 grams. At this point its body water was extremely depleted. The concentration of chloride in the plasma was 332 mgm. per 100 cc. and the hematocrit value was 52 per cent. The concentration of



sodium in the plasma was 317 mgm. per 100 cc. (normal 372 mgm. per 100 cc.). Daily intraperitoneal injections of Ringer's solution were started at this point. Immediate clinical improvement was evident. The body weight increased steadily. One per cent salt was added to the diet the following month. After three months this bird exceeded its original body weight by 30 grams. It was kept in good physical condition, as evidenced by the fact that it started a period of egg laying about five months after the operation. At the beginning of the ninth month of observation it was placed on the basal diet without added salt for four months (fig. 3). At the end of this time its body weight was 960 grams. The concentration of chloride in the plasma was 328 mgm. per 100 cc. and the hematocrit value was 54 per cent. One per cent salt then was added to the diet. The response was immediate and rapid. After six weeks the bird had regained its original body weight.

We performed numerous similar experiments which demonstrated that the responses just described were characteristic. Birds that have exteriorized ureters may be kept indefinitely and in excellent health if due attention is paid to chloride requirements. Many of our birds entered on prolonged periods of egg production. Bird 9 was still living and in excellent health nearly eighteen months after operation.

*Water Balance.* Many of the early workers noted tremendous increases of consumption of water after the construction of an artificial anus or after hepatectomy. If absorption from the cloaca occurs in significant amounts, one would expect an increase of consumption of water to follow interference with the normal anatomic relations. Therefore, we studied this point with a great deal of care.

Birds that have not been operated on consume a variable amount of water. Their twenty-four hour intake ranges from 0.070 to 0.175 cc. of water per gram of bird. Therefore, it was necessary to study the water intake and weight fluctuations of the same birds before and after surgical procedures.

There was a temporary increase of the water intake of some but not of all birds that had artificial anuses (tables 2 and 3) immediately after the operation but the intake when increased returned to the control quantity a short time later.

After exteriorization of the ureters, five birds of the 150 in this group increased their intake of water four or five times the basal value. As a rule, however, the increases were not more than a few cubic centimeters (tables 4 and 5). Some birds increased their intake of water intermittently. When permitted to drink salt water, the birds nearly always but not invariably increased their total intake of fluid. When birds that had exteriorized ureters were fed a diet without added salt, they always increased their consumption of water (table 6). The water intake was often double that of the period when salt was added to the diet.

For purposes of studying the normal volume of urine of birds that had exteriorized ureters we necessarily used birds receiving adequate salt intake (1 per cent sodium chloride in the food). Under these conditions birds that had exteriorized ureters and birds that had artificial anuses behaved exactly alike. The volume of urine varied from 50 to 180 cc. or 28 to 70 per cent of the water intake for the day (tables 1 and 7). This percentage of the intake was fairly constant for any given bird.

TABLE 2

*Daily consumption of food and water and fluctuation of weight in chicken 2 before and after creation of an artificial anus; 1 per cent salt in diet*

DATE, 1940	AVERAGE DAILY WEIGHT	AVERAGE DAILY WATER INTAKE	AVERAGE DAILY FOOD INTAKE	GM. FOOD GM. BIRD	CC. WATER GM. BIRD	CC. WATER GM. FOOD	COMMENT
8-10 to 8-14 8-16	gm. 2,578	cc. 270	gm. 143	0.055	0.105	1.89	Artificial anus created. Blood Cl: 394 mgm. per 100 cc. Hematocrit reading: 27 per cent
8-17 to 8-23	2,338	250	55	0.024	0.107	4.55	
8-24 to 8-30	2,388	236	113	0.047	0.099	2.09	Hematocrit reading: 32 per cent

TABLE 3

*Daily consumption of food and water and fluctuation of weight in chicken 3 before and after creation of an artificial anus; 1 per cent salt in diet*

DATE, 1940	AVERAGE DAILY WEIGHT	AVERAGE DAILY WATER INTAKE	AVERAGE DAILY FOOD INTAKE	GM. FOOD GM. BIRD	CC. WATER GM. BIRD	CC. WATER GM. FOOD	COMMENT
6-17 to 6-23 6-25	gm. 1,613	cc. 213	gm. 93	0.058	0.132	2.29	Artificial anus created
6-26 to 7-2	1,596	238	82	0.051	0.149	2.90	
7-3 to 7-9	1,633	300	58	0.036	0.184	5.17	
7-10 to 7-15	1,542	115	40	0.026	0.075	2.88	Blood Cl: 406 mgm. per 100 cc. Hematocrit reading: 35 per cent

TABLE 4

*The daily consumption of food and water and fluctuation of weight in chicken 4 before and after exteriorization of the ureters; 1 per cent sodium chloride in diet*

DATE, 1940	AVERAGE DAILY WEIGHT	AVERAGE WATER INTAKE	AVERAGE FOOD INTAKE	GM. FOOD GM. BIRD	CC. WATER GM. BIRD	CC. WATER GM. FOOD	COMMENT
5-6 to 5-10 5-16	gm. 1,317	cc. 231	gm. 106	0.080	0.175	2.18	Ureters exteriorized
5-17 to 5-23	1,180	210	70	0.059	0.178	3.00	
5-25 to 5-31	1,255	235	89	0.071	0.187	2.64	
6-18 to 6-23	1,207	122	44	0.036	0.101	2.77	

The amount of water in the feces varied from 5 to 25 per cent of the water intake. This figure, too, was fairly constant for any given bird.

Bird 1 (table 1) may be used to illustrate the calculation of the water balance.

From August 21 to August 26 inclusive, this bird ingested 1,864 grams of food and water. It eliminated 1,078 grams of feces and urine in this period. The difference of these two figures is 786 grams. However, 38 grams of body weight was gained by the bird during the six days. Therefore,  $748/6$  gives 124.7 grams per day lost by way of the respiration (that lost through the skin is doubtless negligible). Values ranging from 110 to 144 grams may be found by similar calculations from the data given for other birds.

TABLE 5

*The daily consumption of food and water and fluctuation of weight in chicken 5 before and after exteriorization of the ureters*

DATE, 1940	AVERAGE DAILY WEIGHT	AVERAGE WATER INTAKE	AVER- AGE FOOD INTAKE	AVERAGE NaCl INTAKE, 0.2%	GM. FOOD GM. BIRD	CC. WATER GM. BIRD	CC. WATER GM. FOOD	COMMENT
4-1 to 4-9 4-16	gm. 1,568	cc. 155	gm. 69	cc. 	0.044	0.099	2.25	Ureters exteriorized
4-20 to 4-26	1,507	106	78	129	0.052			
4-27 to 5-4	1,504	178	69		0.046	0.118	2.58	Blood Cl: 400 mgm. per 100 cc. Hema- tocrit value: 38 per cent
5-6 to 5-13	1,523	199	74		0.049	0.131	2.69	

TABLE 6

*The daily consumption of food and water and fluctuation of weight in chicken 6 before and after exteriorization of the ureters; no salt added to the diet*

DATE, 1941	AVERAGE DAILY WEIGHT	AVERAGE WATER INTAKE	AVERAGE FOOD INTAKE	GM. FOOD GM. BIRD	CC. WATER GM. BIRD	CC. WATER GM. FOOD	COMMENT
1-27 to 2-3 2-4	gm. 2,209	cc. 100	gm. 78	0.035	0.045	1.28	Ureters exteriorized. Blood Cl: 400 mgm. per 100 cc. Hematocrit value: 40 per cent
2-14 to 2-20	2,080	213	48	0.023	0.102	4.44	
2-21 to 2-26	2,042	250	70	0.034	0.122	3.57	

In calorimetric studies on hens, Barott and associates found the water eliminated by way of the respiration to be  $2.8 \pm 0.1$  mgm. per hour per gram at the basal level and  $3.1 \pm 0.1$  mgm. per hour per gram during eight hour periods between 2 p.m. and 10 p.m. These figures give 154 cc. at the basal level and 171.1 cc. for the eight hour period as twenty-four hour values for a bird of 2,300 grams.

*Withdrawal of Drinking Water from Birds That Had Exteriorized Ureters.* The

TABLE 7

*Water balance study on chicken 7 which had exteriorized ureters; 1 per cent salt added to the diet*

DATE, 1940	BODY WEIGHT	FOOD IN-TAKE	WATER INTAKE	WET WEIGHT FECES	DRY WEIGHT FECES	WATER FECES	URINE VOLUME	pH OF URINE	URIC ACID	PLASMA Cl.	HEMA-TOCRIT VALUE
	gm.	gm.	cc.	gm.	gm.	cc.	cc.		gm./100 cc.	mgm./100 cc.	
8-21	1,418	61	220	35	5	30	100	6.71	1.56	412	40
8-22	1,419	85	255	47	12	35	112	7.29	1.09		
8-23	1,484	90	245	74	13	61	107	6.64			
8-24	1,480	96	250	63	4	59	144	6.95	1.36		36
Averages....	1,450	83	242	54.7	8.5	46.2	115.7				

TABLE 8

*Collection, by use of the glass cannula, of urine from normal bird (no. 8) prior to operation; 1 per cent salt in diet*

TIME	URINE VOLUME	COMMENT
	cc.	
11:14		Clear, watery urine
11:28	7.5	Milky urine
11:30		White shreds appear
11:40		Bird moderately disturbed
11:43		Urine less cloudy
11:53	3.0	Urine clear as water
12:00		Urine slightly cloudy
12:10		Urine less turbid; bird moderately disturbed
12:30		Urine clear as water
12:32		Urine cloudy again, with chunky material
1:00		Anuric for past 28 minutes; asleep
1:10		Flow increased to 2 drops per minute
1:20	2.5	Collection ended
	13.0	Total volume of urine collected in period
1:15		Two days after foregoing collection
1:35	3.0	Urine slightly turbid; 3 drops per minute
1:41		Urine very milky
1:44		Urine clear as water; bird struggles
1:47		Urine cloudy
1:53		Urine clear as water; bird disturbed
1:58		Urine clear as water; 4 drops per minute
2:00		Urine slightly turbid; 1 drop per minute
2:19		Urine thick white; 1 drop per minute
3:10	6.5	Anuric for last 14 minutes Collection ended
	9.5	Total volume of urine collected in period

importance of absorption of water from the cloaca or rectum should be demonstrated by the ability of the fistulous bird to withstand a prolonged lack of water. Accordingly, birds that had exteriorized ureters were refused water and their loss of weight was compared with that of control birds under the same situation (fig. 4). Losses of weight should be compared during the first few days of observation while the birds are normally hydrated. Total losses of weight during the first three days of observation for the controls were 74, 78, 99, 102, 113, 120 and 121 grams. Simultaneous losses for the fistulous birds were 122, 132, 137 and 189 grams.

*Urine Collections From Hens Before Surgical Procedure.* Hester and associates, using the funnel technic of collection of urine (5), observed a primary phase of copious flow of urine followed by a phase of scant flow of urine. They showed that nervous influences are among the factors which determine the quantity of flow of urine. We have extended these observations and confirmed the conclusions of the previous work. The cannula described in this paper for collecting urine permitted a longer period of observation than did the funnel technic. A typical experiment will now be presented briefly.

One of the birds secreted 7.5 cc. of urine in the first fourteen minutes of collection. In the next twenty-five minutes it secreted 3 cc. of urine, and in the following eighty-seven minutes it secreted 2.5 cc. of urine. In the primary phase the urine secreted was clear and watery while the urine collected during the secondary phase was reduced in volume and contained solids in varying amounts. On a different day the same bird secreted 3 cc. of urine in the primary phase of twenty minutes. In the following period of ninety-five minutes it secreted 6.5 cc. of urine (table 8).

COMMENT. It is clear that studies on water consumption are not adequate criteria for establishing the concept of absorption from the cloaca.

The excessively large estimates placed on the urine volume (600 to 1,500 cc. per 24 hrs.) by some previous workers may be accounted for in the following ways. Wiener and Milroy were studying birds after removal of the liver. Minkowski gave a satisfactory explanation for the increased water intake after this operation by pointing out that more blood was required to go to the kidney, which should result in increased excretion of urine.

Davis found that anesthesia caused a greatly increased flow of urine and he pointed out that calculations of the daily volume of urine under such conditions were erroneous. The observations of Hester and associates of a diuretic phase of secretion of urine indicate an additional reason why a false impression of the daily volume of urine would follow if calculations were based on the data obtained from short collection periods.

Davis suggested that the bird depends on absorption from the cloaca for the conservation of various urinary constituents, including creatine, urea and chloride.

In our studies of hens that had exteriorized ureters, we found that the addition of sodium chloride to the dietary would maintain them in excellent health for indefinite periods. Because of the latter fact and because severely deficient

birds could be restored to normal by giving salt alone, we conclude that reabsorption of electrolytes and water from the rectum is the only mechanism of importance in the normal economy of the chicken.

When drinking water is withheld, fistulous birds lose weight more rapidly than controls. This observation constitutes suggestive evidence for the importance of the rectum in water conservation. Although we have these facts at hand, and although we have demonstrated the importance of absorption of chloride, our data do not permit us to make a final statement concerning the magnitude of these absorptions. Further investigation will be necessary to determine the type of work involved in the process of absorption.

Birds that have exteriorized ureters lose weight rapidly immediately after the operation. But fistulous birds that have been maintained on salt for long periods lose weight more slowly when placed on a diet without added salt. One bird lost nearly half its body weight in six weeks immediately after exteriorization of its ureters, but four months were required (fig. 3) to produce the same loss of weight after it had been carried on adequate salt intake for six months. Thus it would appear that there is some adaptation to the new condition.

Korr<sup>(7,8)</sup> presented good evidence to show that absorption does not occur from the cloaca. We do not have data on this point, since our birds that had artificial anuses were given 1 per cent salt in the diet. This amount of salt is sufficient to maintain the fistulous bird and therefore would be ample for birds that had artificial anuses.

Korr found that 60 to 100 cc. of water, as a maximal amount, was excreted by the kidney for every gram of uric acid. These figures were based on determinations of total nitrogen of relatively dilute urines. From the data of Szalágyi and Kriwuscha we calculate 100 to 200 cc. of urine water for each gram of total nitrogen (chickens). We found 30 to 165 cc. of urine water to be excreted with each gram of uric acid. Since all our birds were ingesting normal amounts of water, the volumes of urine were doubtless normal.

#### SUMMARY

The daily consumption of food and water and weight fluctuations of normal birds were studied. Following this control period, artificial anuses were created in some of the birds while the ureters of others were exteriorized.

There was an increase of the water intake of some birds that had artificial anuses immediately after the operation but the intake returned to the control quantity a short time later.

When the fistulous birds were given 1 per cent salt in the diet, occasional increases of water intake were noted. If salt was not added to the diet, an increase of water intake always occurred. We do not have any satisfactory explanation for this paradox.

When salt was omitted from the diet of fistulous birds, weight losses were pronounced and eventually death occurred, with evidences of extreme depletion of body water. However, these effects were prevented or ameliorated by the

addition of 1 per cent salt to the diet and this amount of salt was sufficient to maintain the birds indefinitely.

Fistulous birds lose weight more rapidly than controls when drinking water is withheld, indicating that normally some water is reabsorbed from the rectum.

When adequate salt intake was assured, birds that had exteriorized ureters behaved exactly like those that had artificial anuses. The normal volume of urine varied from 50 to 180 cc. per day. The amount of water lost in the feces was usually about 10 per cent of the water ingested for the day. Vaporization from the lungs accounted for 110 to 144 cc. of water per day.

Each gram of uric acid eliminated was accompanied by 30 to 160 cc. of water.

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# THE VASOMOTOR COMPONENTS IN THE VASCULAR REACTIONS IN THE FINGER TO COLD<sup>1</sup>

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The vascular responses in the finger to local chilling have become well-known since their description by Lewis (1) and Grant (2). They usually occur in the following sequence: initial constriction occurs on application of the cold; some 5 to 16 minutes later dilatation occurs while the cold is continued; this reaction has a variable duration being followed by constriction and again by a second reactive dilatation, the cycle being repeated if the cold is continuous; on ending the cold, the vessels dilate rapidly so that the temperature of the previously chilled skin may considerably exceed that of a control finger.

These reactions were elicited also in the toes, ear, tip of nose and chin. Since these skin areas as well as the finger contain a large number of arterio-venous anastomoses, it was suggested that these vessels are the ones responsible for the reactive dilatation to cold.

The dilator reactions during cold persisted after sympathectomy and after degeneration of the sympathetic fibers. They disappeared after degeneration of the sensory fibers but not after section of the latter. This similarity to the histamine flare suggested that the reactive dilatation is elicited by the liberation of H-substance which in turn excites the axone reflex.

This description is based on observational methods which do not clearly indicate the time relations of vasomotor activity to the reactions to cold. It appeared probable that suitable plethysmography would examine these reactions in greater detail and would permit a better correlation between the reactions and the vasomotor reflexes elicited by the cold. It also seemed possible that through plethysmographic analysis one would obtain additional information on the relative participation of arterio-venous anastomoses, small arteries and arterioles, and larger artery trunks (e.g., the digital arteries), in the constrictions and reactive dilatations to cold in the human finger.

**METHODS.** The vascular reactions in the finger to local cold were followed by means of the photoelectric plethysmograph (3). The use of this instrument simplified the mechanical problem of applying cold and of recording simultaneously the changes in volume or in volume pulse. Variations in the latter were considered as indicative of changes in arterial tone.

Cold was applied to the finger in three ways: 1, cold water was circulated about

<sup>1</sup> This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association. Helpful aid has also been received from the Burgess Battery Company, Freeport, Illinois.



the finger; 2, the finger was placed in a bath of chopped ice; 3, cold water was circulated through a copper tube soldered to a thin copper sheath in contact with nearly the entire surface of the finger. In each case the photoelectric plethysmograph was applied to the pad of the finger being chilled.

When the finger was immersed in water, the plethysmograph was protected by cementing a sheet of glass over the openings of the plethysmograph tubes which were passed through a rubber stopper in the wall of the bath. The finger dipped into the bath from above. It was kept in place over the plethysmograph by a piece of sponge rubber cut to fit between the finger and the wall of the bath without exerting significant pressure on the finger. Since the top of the bath was open, there was no mechanical interference with the circulation in the finger.

The copper sheath used to chill the finger was soldered directly to the plethysmograph. This was the most convenient means of chilling the finger. It was effective and provided the same results as the immersion method.

A finger on the same hand and often also one on the other hand served as controls. Temperatures of the pads of the control and experimental fingers were continuously recorded by thermocouples.

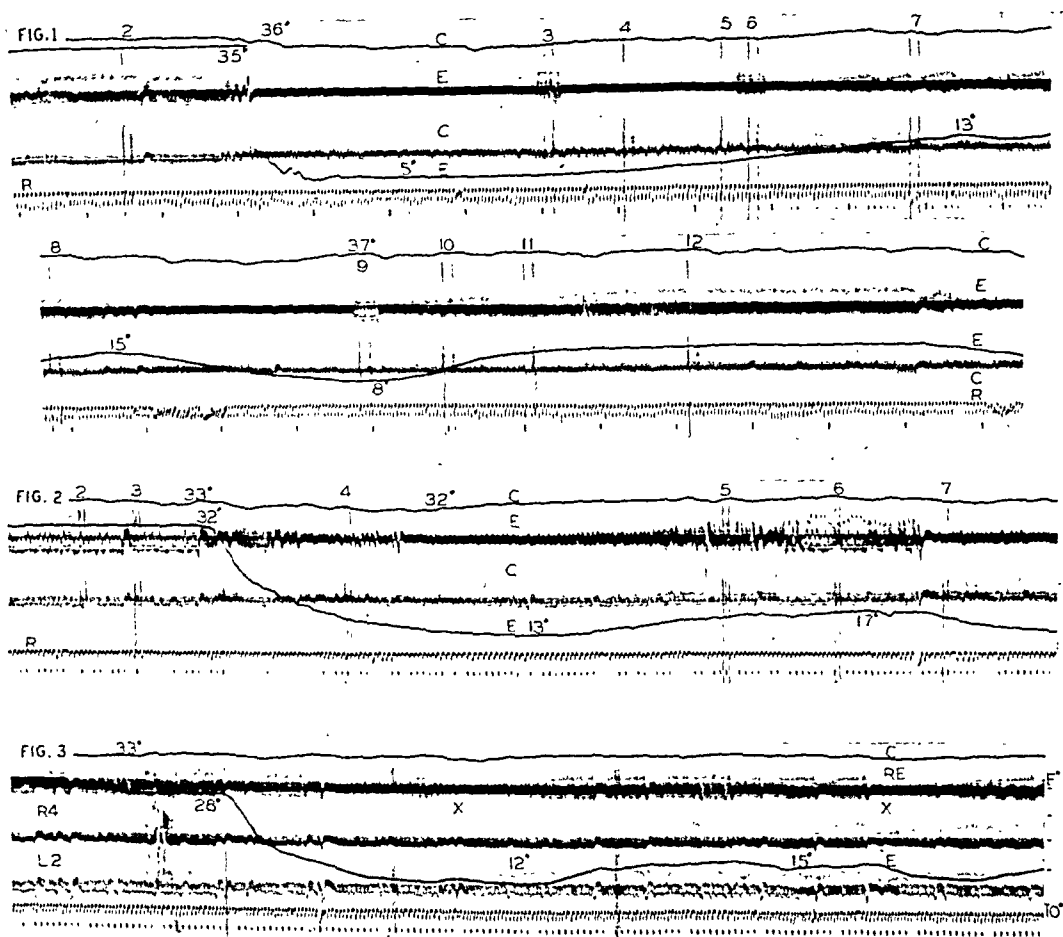
Every effort was made to secure the comfort of the subject who rested easily on a cot in a semi-reclining position. However, in prolonged experiments of this type, the continuation of the same position of the arm often results in numbness in fingers, hand and forearm. As far as we could detect, this did not directly affect the finger blood flow and the vascular reactions. We saw no relation between the onset and the degree of numbness and the finger blood flow.

The subjects were medical students in good health and with normal circulatory systems, unless otherwise noted. Fitting the apparatus to the subject and similar preparations usually required thirty to forty-five minutes during which time the subject was resting on a cot. Hence, a control record of fifteen to twenty minutes came after a considerable rest period so that the subject was probably in good equilibrium with the room climate ( $25^{\circ}$ – $27^{\circ}\text{C}.$ ) before experimenting began. Attainment of equilibrium was favored by the subject having been in the building for several hours before coming to the laboratory.

A serious difficulty with prolonged experiments of the type reported here is that immobilization of the subject results in a slow progressive decrease in the blood flow in the finger. This may not show well in the finger temperatures when the room temperature is in the range of  $25^{\circ}$  to  $27^{\circ}\text{C}.$  The cooling effect of this air temperature on the finger is slight, hence it is possible for a considerable reduction in blood flow to occur without much effect on the finger temperatures. This is more apparent when the flows are high than when they are low. The effects on finger temperature are more obvious at lower air temperatures. This progressive fall in finger flow was noted by Lewis (1) and allowed for in comparing the reactions of the chilled finger with the control after the application of cold had ended. His experiments were carried out at lower room temperatures.

**RESULTS.** *The initial constriction.* On application of cold to the finger (fig. 1–5), immediate and profound constriction occurs not only in the finger being

chilled but also in the control fingers of the same and opposite hands. The constriction in the chilled finger may be immediately complete (figs. 1 and 4) or it may become progressively more intense within a brief period of ten to



Figs. 1-3. Effects of local cold in finger circulation. Records of volume pulses and temperatures of experimental or chilled finger, *E*, and of control finger, *C*. Wave form records taken at triple vertical signals (see following paper). Time in minutes and 5 seconds. Respiration by pneumograph.

Fig. 1. Chopped ice applied at break in temperature record. Cold continued for remainder of record. Amplification temporarily increased at 3, 6 and 9 for wave form records. R.T. 26°C. Male. Age 24. B.P. 112/74.

Fig. 2. Ice water at break in temperature record. Cold continued. R.T. 25.5°C. Male. Age 23. B.P. 120/80.

Fig. 3. Ice water at break in temperature record. Cold continued. R.E.—right index finger (experimental or chilled); R4—right fourth finger; L2—left index finger. R.T. 26°C Male. Age 24. B.P. 120/74.

seventy seconds (fig. 2). The initial constriction in the chilled finger may be brief (60 sec.) or it may last longer, (4 to 15 or more min.). The initial constrictions in the control fingers are usually less intense and of shorter duration. They are due to vasoconstrictor reflexes elicited by the cold since

they appear in all control fingers (of the same and opposite hands) at the time they occur in the chilled finger.

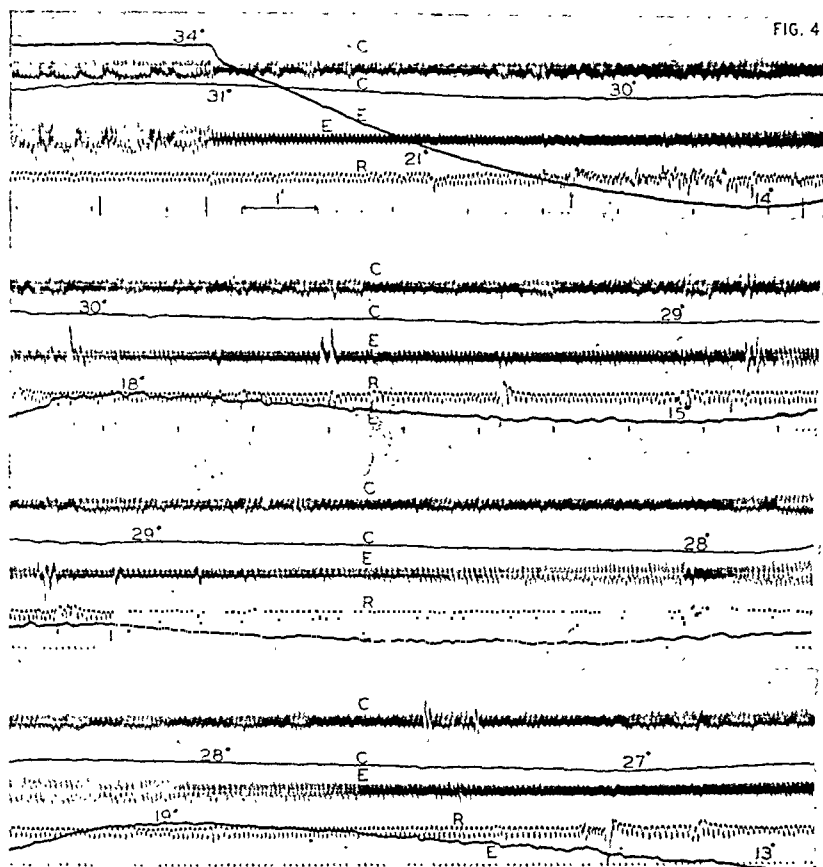


Fig. 4. Experiment illustrating paralysis of vasomotor fibers in the chilled finger during the reaction of dilatation. Cold applied by cold water (4°C.) flowing through a copper applicator. Volume pulses and temperatures of experimental, *E*, and control, *C*, fingers. Cold applied at break in temperature record. Time in minutes and 5 seconds. Respiration by pneumograph. R.T. 25°C. Male Hawaiian. Age 23. B.P. 98/58. Subject dislikes cold very much but does not show cold allergy.

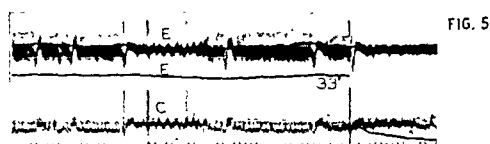


Fig. 5. Comparison of the effects of the cold pressor test (at horizontal bar) and of local cold (ice at break in temperature) on the finger circulations. Volume pulse and temperature records from experimental, *E*, and control, *C*, fingers of same hand. R.T. 26°C. Male. Age 24. B.P. 116/78. Normo-reactor.

The differences in the intensity and duration of the initial constrictions in the chilled and control fingers may be due to a direct local constrictor action of cold on the arteries superimposed on vasoconstrictor reflexes elicited by the cold or to these reflexes acting exceptionally strongly on the chilled finger.

The changes in finger temperature are pertinent to a decision. The initial constriction in the chilled finger may proceed to obliteration of the pad pulse before there has been any large fall in the recorded temperature of the pad of the chilled finger (fig. 4). In some cases, the chilled finger's pad temperatures were as high as 30°C. at the time of complete constriction. It may be argued that the *surface* temperature of the finger tip does not represent the temperatures of the pad arteries whose reactions are being recorded and is therefore not significant as a basis for correlating the temperature drop with the intensity of the constriction. There seems to be no way of eliminating this error since the argument would apply equally well to measurement of the temperature of any part of the finger's surface. The comparison of the finger and forehead reactions to local cold (described in a following paper) suggests that the cooling of the finger arteries proceeds more rapidly than one would infer from the finger tip temperatures. The initial constriction due to a vasomotor reflex would favor swift cooling. Absence of this reflex in the forehead skin would prevent a rapid fall in temperature there. Arterial constriction would then proceed much more gradually.

The fall in temperature may not induce a sustained constriction in the chilled finger (fig. 3). The record of its volume pulses may parallel quite closely the records of the control fingers. Each vasomotor discharge to the latter may be faithfully reflected in the record from the chilled finger. Yet, the constrictions in the latter tend to be more prolonged (fig. 3 at X). It is difficult to believe that as a result of chilling, the vasomotor discharges would be selectively prolonged with respect to the cold finger. Two alternatives remain: either the vasoconstrictor effect is prolonged by the direct action of cold on the arteries or the lower temperature prolongs the life of the chemical mediator. Observations recorded below on the nature of the reactive dilatation bear on this question.

That the onset of the initial constriction is exclusively due to a vasomotor reflex is indicated by several observations: 1. The constrictor response of the sympathectomized toe (in a hypertensive patient) to cold resembled that of the forehead skin. Constriction developed gradually as the toe temperature fell. 2. Variability in the participation of vasoconstrictor reflexes in the constriction to cold was interestingly illustrated in a young man in whom a peripheral vascular disorder was suspected (skin from mid-calf to toes became fiery red then cyanotic on standing). The big toe showed, on first observation, the absence of vasoconstrictor reflexes to such procedures as psychic stimuli and deep breaths which were very effective in inducing constriction in the finger. Application of cold to the toe failed to elicit the initial constriction there. The cold stimulus was effective in eliciting vasoconstrictor reflexes since the finger constricted markedly when the cold was applied to the toe. As the temperature of the latter fell, the toe vessels constricted gradually but not to obliteration of the volume pulse. A reactive dilatation followed this constriction. Three days later during which time there was no therapy, the experiment was repeated. The toe now showed very effective vasoconstrictor reflexes and responded to a similar cold application with a typical immediate profound constriction which also occurred in the toes of the other foot. The toes were warm (about 31°C.)

and showed large volume pulses on each day so that the differences in behaviour to the cold and other stimuli were not due to differences in the blood supply. We have not had the opportunity to study this patient further. 3. The possible hypersensitivity of the finger arteries to cold in Raynaud's disease offered opportunity to compare the constrictions elicited by local cold with and without participation of vasoconstrictor reflexes (as indicated by the reaction of the control finger). Two experiments were done on a woman (age 40) in the earlier stages of the disease. In the first experiment, water at 15°C. was passed through the cold applicator attached to the right index finger. Constriction began very slowly and gradually about a minute later in this finger. This constriction became progressively more intense, reaching its maximum about fourteen minutes after the onset of cold. It was maintained for the duration of the cold (25 min.). There was no accompanying constriction at any time in the control finger. Both fingers were free of "spontaneous" waves during the entire observation. This subject gave an intense response in the fingers to the "cold pressor test". The absence of any indication of vasomotor participation in this response to local cold points to a direct action of cold on the finger arteries. This response was analogous to that occurring in the forehead. Repetition of the experiment at a later date with colder water (8°C.) showed the usual vasoconstrictor reflexes on the application of cold. The following reactive dilatation was remarkable for its size and duration but did not result in a subsequent swelling of the finger. 4. Similar experiments on another case of Raynaud's disease on which a unilateral cervical sympathectomy had been done, illustrated most convincingly that the absence of the vasoconstrictor reflexes in the chilled finger delays the onset of the constriction to cold and results in a more gradual development of the constrictor response.

It is interesting to note that these initial constrictions in the control fingers may be as intense as those elicited by plunging the opposite hand into ice-water (fig. 5). The constrictions may be better maintained in the latter case. Not all subjects give maximal constrictions in the finger on applying cold to it or to its neighbor, nor do they do so when the "cold pressor test" is done. It would be of interest to attempt a comparison of the finger constrictions in the normo- and hyper-reactor groups of subjects on the local application of cold and on making the "cold pressor test."

As the application of cold continues, the vessels in the control fingers usually relax partially while those in the chilled finger usually constrict still more as its temperature falls. That vasomotor activity in the control fingers continues at a higher level is indicated by the reduction in the average amplitude of the volume pulses, by greater frequency of the "spontaneous waves" as compared with the control period, and by the tendency of the control finger temperatures to fall.

It is difficult to account for the increased vasomotor activity on a thermosensory basis, for it continues after the chilled finger has become numb. Certainly the extremely small return of chilled blood from a single cold finger can have no direct central effect on the temperature regulating mechanism. The

vasoconstrictor effects of cold may be elicited without sensation. Thus, the blood pressure raising reflex from the cold pressor test was obtained in a patient showing hysterical anesthesia to cold, heat and pain in the cutaneous areas supplied by spinal segments (4). The patient was unaware of the application of ice to the forearm and hand; yet a significant rise in blood pressure occurred. Similarly, reflex vasodilatation may be obtained by warming an occluded limb in which thermal sensation is absent (5). The thermal sensations are therefore unnecessary to the appearance of vasoconstrictor reflexes in response to chilling or warming the skin. Neither is pain essential to their continuance.

*The "reactive dilatation"*. Sooner or later (3 to 8 min. or more), after the beginning of the cold application and while the cold is continued, the constricted chilled finger vessels slowly and progressively dilate as indicated by the rise in the finger's temperature and by the increase in its volume pulse. The increase in the latter is usually obvious for several minutes while the temperature is still falling (fig. 4—upper record). This progressive dilatation is often limited to the chilled finger although it is also frequently preceded by dilatation in the control finger. This suggests that a decrease in vasomotor activity may be a factor in the dilatation.

*The relation of vasoconstrictor reflexes to the reactive dilatation.* Our experiments have permitted an examination of this relation. In some cases, vasoconstrictor reflexes were ineffective in the chilled finger during the "reaction," while in other experiments the opposite was true. In still other instances, reflex vasoconstrictor effects in the chilled finger developed slowly and tended to be prolonged. There were no systematic differences in the magnitude of the reactive dilatation in different subjects in relation to the absence of or presence and degree of vasoconstrictor reflexes in the chilled finger.

During the reactive dilatation in the cold finger, heightened vasomotor tone and spontaneous constrictions (vasomotor in origin) in the control fingers may be without any measurable effects on the chilled finger (fig. 4) even when its volume pulse is well above the control level. The "reaction" may develop into a large dilatation while high vasomotor tone continues in the control finger. Deliberately eliciting vasoconstrictor reflexes during the "reaction" as by plunging the opposite hand into ice-water, or by effective psychic and auditory stimuli, or by a deep breath, were also ineffective in the chilled finger in these instances, although strong constrictions were obtained in the control fingers. We interpret such results as indicating a temporary paralysis of the vasomotor fibers in the chilled fingers.

In searching for reasons for this "paralysis," we have examined its correlation with the temperature of the finger pad without success. "Reactions" occurred at finger pad temperatures between 5° and 23°C. "Paralysis" was seen with the finger pad temperatures as high as 29°C. as the result of a large "reaction" and was absent at 5°C.

The "paralysis" tended to disappear as the "reaction" developed (fig. 4—lowest record), but it might still be present when the volume pulse was again as large as or larger than during the control period. The "paralysis" might

continue throughout a "reaction" and the latter would then subside gradually into another prolonged constriction without evidence of vasoconstrictor activity (fig. 4—first and second "reactions"). The presence of "paralysis" bore no systematic relation to the size of the "reaction". But high vasomotor activity in the control fingers did limit the "reactions" in the cold finger if the vasoconstrictors were still active there (figs. 1 and 2), the reactive dilatation usually being ended by a vasoconstrictor discharge to all fingers. This point probably would be missed in temperature records since a fleeting constriction in the control finger too brief to influence noticeably the finger's temperature may be the signal for the onset of another prolonged constriction in the experimental finger (figs. 1 and 2). Continuous records of the volume pulse or of finger volume are required to illustrate the point.

The first constriction which occurs during the reactive dilatation may not pass into another prolonged constriction with a marked fall in finger temperatures. Several constrictions may occur before this happens (figs. 1 and 2). But it is usually true that the first considerable constriction in the control finger during the "reaction" ends the latter. In this sense, the duration of the "reaction" is limited by vasomotor activity.

The events elicited by the initial application of cold are now repeated. The constriction in the cold finger lasts for a variable period (4 to 8 min.). It is followed by another reactive dilatation which also is ended by a vasoconstrictor discharge (fig. 1). Several such cycles may occur providing the patience of the subject permits the prolonged sitting which is required.

The vasomotor "paralysis" is not always complete and it appears to pass through transitional stages which show in a constrictor response which develops gradually rather than in the abrupt manner usually true for the control fingers (last record of fig. 5); the response may also be much less intense and may last longer than in the control fingers. As the reactive dilatation proceeds, the constrictor response in the experimental finger becomes more and more like those in the control fingers: abrupt, intense, brief or long as may chance to be the case in the latter. One is reminded of the possibility of a chemical mediator being liberated at a slower rate, acting more slowly, and being more slowly eliminated due to the lower temperatures. Or, these effects may be due to the direct effects of the lower temperatures on the smooth muscle of the arteries.

Not all experiments progress in the manner described above. Some normal subjects fail to give prolonged constrictions to chilling the finger (fig. 3). Such subjects' fingers appear to have excellent blood supplies (judging by the amplitude of their finger volume pulses) which apparently prevent most effectively the fall in finger temperature required to bring on a prolonged constriction. After an initial constriction of brief duration in the experimental and control fingers, circulatory conditions, except for the lower temperature of the chilled finger, are restored to control levels and remain so. Inspection of the records in these cases often permits no distinction between the control and the experimental periods. These subjects may experience pain from the chilling. However, their discomfort is short-lived. In this respect they do not differ from the

subjects who present the cycle of prolonged constriction followed by the reactive dilatation.

*Recovery.* The period of recovery from the exposure to cold proceeds as described by Lewis (1). A reactive dilatation occurs in the chilled finger if there has been constriction from the cold. This "reaction" may not increase the flow above the control level but the fact that it is occurring is indicated by comparison of the pulses in the experimental and control fingers. The pulses in the former are larger.

There are several points which bear on the reasons for this dilatation during recovery in the experimental finger. First, it occurs even when there is a high vasomotor tone in the control fingers as indicated by the reduction in their volume pulse. Second, at this time the vasoconstrictor reflexes are as effective in the experimental finger as in the control fingers. The constrictions are as abrupt, and as intense in the experimental finger as in the control. Still the vessels in the former remain widely dilated while those in the control fingers are partly constricted. The differences in the circulatory situations may be quite impressive at times.

*COMMENT.* Interpretation of the experiments described above is concerned with two familiar phenomena: 1, the constrictor effect of local cold; 2, the reactive dilatation which follows while the cold application is continued and which is commonly recognized in the sensations of throbbing and of warmth in the exposed fingers.

1. The initial constriction in the skin of the finger due to local application of cold results from vasomotor reflexes elicited by the cold. This is indicated by the simultaneous constrictions occurring in the control fingers. Although differences in the intensity of the initial constriction in the experimental and control fingers are no more striking than those seen in responses to other stimuli, they are regularly exhibited. We take this to mean that an additional direct effect of cold may be superimposed on the vasoconstrictor reflex. However, in many instances there is not enough time for the direct effect of cold to account for the intensity of the constriction. Hence, the reflex effects appear to be dominant in the initial constriction. The direct effects of cold develop more slowly as illustrated in the reactions in the forehead and in cases of hypersensitivity to cold.

The vasoconstrictor tone may continue at a high but variable level in the control fingers throughout the cold application. This is probably a factor in maintaining constriction in the cold finger, for relaxation in the control fingers often precedes and accompanies the reactive dilatation in the cold finger.

2. It is the reactive dilatation in the chilled finger which is difficult to explain. The following facts must be kept in mind in examining the mechanism of its production:

a. The reactive dilatation is definitely not due to vasomotor paralysis for it occurs when the vasoconstrictors can be shown to be effective in the chilled finger. However, decreased vasoconstrictor tone in the control fingers may precede or accompany the reactive dilatation in the experimental finger. This



statement does not deny the occurrence of vasoconstrictor paralysis in the chilled finger. Abundant evidence was obtained to show such paralysis during the dilatation and also during the latter part of the period of constriction induced by the cold. But the paralysis is not essential to the reactive dilatation.

b. The reactive dilatation ends either abruptly due to a powerful vasoconstrictor discharge to the fingers or gradually and progressively. The latter is usually the case when vasoconstrictor paralysis continues throughout the period of dilatation. In either case, the resulting constriction may be as prolonged as the first one in response to the cold. The cycle of constriction and dilatation may then recur repeatedly.

c. The reactive dilatation occurs at the end of the cold application during the recovery period despite a depressed circulation in the control fingers and despite vasoconstrictor reflexes being effective in the experimental finger.

For these reasons we consider that the reactive dilatation develops independently of the vasomotor system. Nevertheless, it is subject, in those cases where the vasoconstrictor fibers are not paralyzed by the cold, to modification by the vasomotor system. This position is essentially the same as that of Lewis (1). It has been arrived at by observational techniques permitting a more detailed inspection of the phenomena.

The data presented above do not indicate which components of the finger's vascular system are involved in the constriction to cold and the subsequent reactive dilatation. Analysis of this problem has compelled an extension of the experiments described here and the description of the data in a following paper.

#### SUMMARY

The vascular reactions in the finger to chilling have been examined by means of the photoelectric plethysmograph. Analysis of these reactions was concerned with the role of the vasomotor reflexes.

The initial immediate constriction on application of cold is due to vasoconstrictor reflexes on which is superimposed somewhat later the direct constrictor action of cold. Evidence:

1. Accompanying constriction occurs also in the warm control fingers of the same and opposite hands, but the constriction is usually more intense in the chilled finger.

2. If a vasoconstrictor reflex is not elicited in the control fingers by an application of moderate cold, the constriction in the chilled finger occurs in a gradual progressive manner, as in the forehead skin, due to the direct constrictor effect of cold on the vessels.

The reactive dilatation, which follows in the chilled finger within three to eight minutes after the application of cold, occurs independently of the vasomotor system. Evidence:

1. The dilatation may be limited to the chilled finger and may occur there when the vasoconstrictor tone is high in the control fingers.

2. Vasoconstrictor reflexes were elicited in the chilled finger during the reac-

tive dilatation in some experiments, while in other instances definite evidence of vasoconstrictor paralysis in the chilled finger was obtained.

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# THE REACTIONS OF THE DIGITAL ARTERY AND MINUTE PAD ARTERIES TO LOCAL COLD<sup>1</sup>

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A. *The selective effects of cold on the digital artery and minute pad arteries.* There are several lines of evidence which suggest that the effects of cold on the finger's circulation are selective with respect to the portions of the finger's arterial tree involved in the reactions. First, it has been shown that the larger arteries of the hand and finger (dorsal metacarpal arteries and digital arteries) and also the radial artery, do not ordinarily participate in the vasoconstrictor reflexes which are elicited by such stimuli as startle, a deep breath, or immersion of the opposite hand in ice water, although these stimuli produce marked constrictions in the terminal arteries of the pad (1, 2). These results were interpreted to mean that the vasoconstrictor discharges were selective with respect to those portions of the arterial tree in the hand which were excited to contract.

Second, Lewis (3) developed a strong argument from indirect evidence that the circulatory deficiency in Raynaud's disease resulted from a spasm of the digital arteries and that it was not primarily due to constriction of the terminal vessels. This position implies a specific selective action of cold on the digital artery trunks of these patients, an interpretation which is compatible with the selective character of the vasoconstrictor discharges to the patient's arterial circulation.

Third, Grant's direct observations on the vessels of the rabbit's ear (4, 5) showed that the entire arterial tree of the ear constricted when cold was applied, the maximal effect occurring in the terminal arteries and arterioles. The selective action of cold in his experiments was exerted on the arterio-venous anastomoses and was shown in the reactive dilatation when the arterio-venous anastomoses were the first vessels to open, the central artery of the ear remaining constricted until the rise in temperature caused these vessels to relax again. It was inferred from these experiments that similar reactions occurred in the human finger. The finger's temperature curves during the application of cold and the presence of arterio-venous anastomoses in the finger skin suggested that these vessels were the ones which were responsible for the reactive dilatation in the finger as well as in the rabbit's ear (6). This need not be the case, however. It is quite possible that the reactive dilatation occurs also in the minute arteries and arterioles of the pad and not only in the arterio-venous anastomoses.

<sup>1</sup> This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association. Generous assistance has also been received from the Burgess Battery Company, Freeport, Illinois, and is gratefully acknowledged.

The experiments described below are concerned with the following questions:

1. What is the relative participation of the terminal pad vessels and of the digital artery trunks in the vasoconstrictor reflexes elicited in the chilled finger?
2. Are there differences in the direct effects of cold on the digital artery and the terminal pad vessels?
3. Do the digital artery trunks participate in the reactive dilatation?

**METHODS.** The experiments have been carried out with the same cooling methods which were described in the preceding paper (7). Records of the volume pulses of the pad and of the digital artery (2) were taken with the photoelectric plethysmograph. Considerable difficulty was experienced in obtaining valid records of the digital artery volume pulses over long periods of time. Slight shifts in the position of the finger will alter the recorded amplitude of the digital artery pulses. These shifts are difficult to prevent. Immobilization of the finger with sponge rubber pads which were cut to support the finger in a fixed position was partially successful in maintaining a constant relation between the digital artery and the plethysmograph. Numbness of the finger during the application of cold prevents the subject from controlling its position. These sources of error probably invalidate the comparison of the amplitudes of the digital artery pulses during recovery and control periods but do not seem to prevent comparison of digital artery and pad artery behaviour during the application of cold.

The amplitudes of the pulses in the pad and digital arteries are considered in these experiments as reflecting corresponding changes in the state of constriction or of dilatation of the corresponding vessels. It is appreciated that the considerable changes in the finger's arterial dynamics in these experiments may modify this relationship. It is also possible that the approximately quantitative relationship shown to exist between the finger volume pulses and the finger blood flow (8) may not hold, particularly during the reactive dilatation, if arterio-venous anastomoses are principally responsible for this reaction.

The temperature of the skin surface as recorded by thermocouples in these experiments probably show only the directional changes in the temperature of the deeper tissues. No attempt was made to correlate in a quantitative manner the arterial reactions with the changes in the temperature of the skin surface. Nevertheless, useful qualitative correlations became apparent.

**RESULTS.** The effects of cold application on the finger temperature varied with the method used to chill the finger and with the state of the circulation in the subjects finger. The rapidity, extent, and duration of the fall in the finger's temperature determined the resultant circulatory effects with respect to the parts of the arterial system of the finger which participated in the reactions and also the extent of their participation.

The relationship of the arterial reactions in the finger to the fall in finger temperature is illustrated in the sequence of figures 1 to 4. With only a slight fall in finger temperature (fig. 1), there is an increase in vasomotor activity (elicited by the cold stimulus) which shows in increased frequency of "spontaneous waves" and reduction in the average amplitude of the pulses in the

pad vessels but which is without effect on the amplitudes of the digital artery pulses. This absence of a reflex vasoconstrictor effect on the digital artery is in

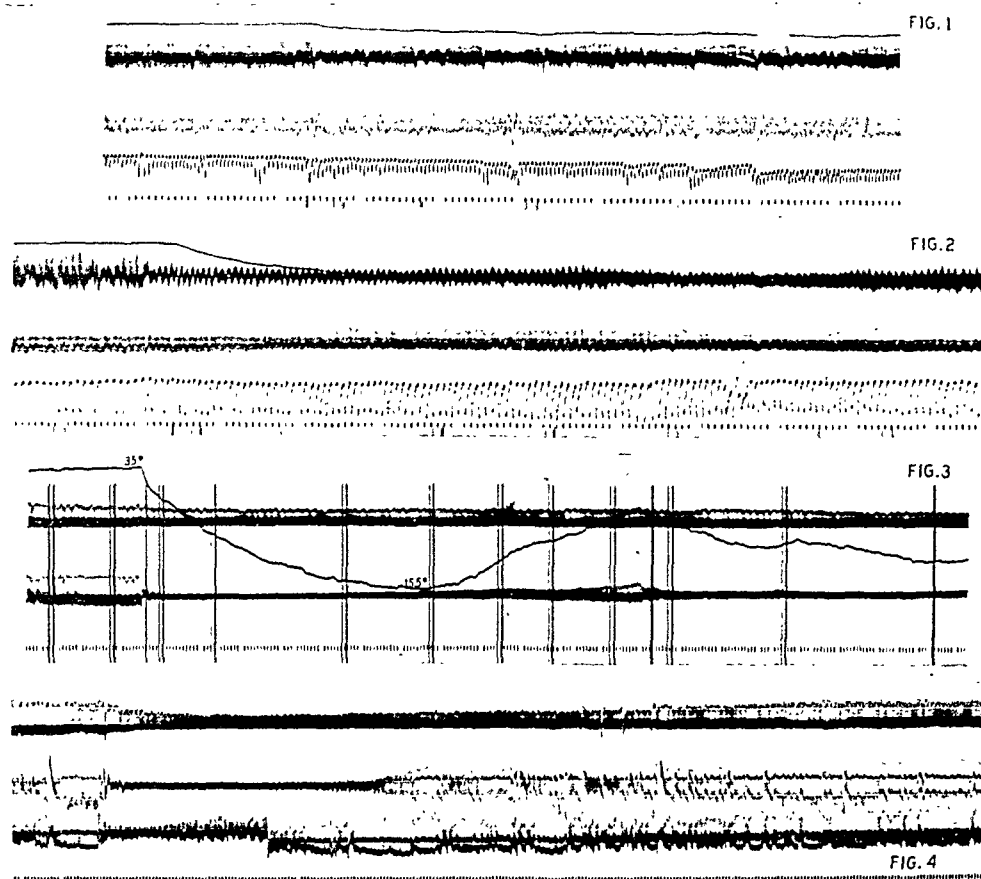


Fig. 1. Reactions of finger arteries to cold which was applied to the whole finger by means of a specially designed copper applicator. Cold applied at arrow and continued for the remainder of the record. Records from above, downwards: finger pad temperature, pad volume pulse, digital artery volume pulse, respiration, time in intervals of five seconds. May.

Fig. 2. Cold by copper applicator to finger at arrow and continued for remainder of record. Top record of finger pad temperature, next of pad volume pulse, next of digital artery volume pulse, respiration, and time in intervals of five seconds. June.

Fig. 3. Cold applied at arrow by copper applicator and continued to end of record. Upper volume pulse record from digital artery, lower from pad. Temperature record from pad. Time in intervals of five seconds. Vertical signals indicate moments of sampling of wave forms on fast kymograph. November.

Fig. 4. Finger chilled by chopped ice at first signal. Ice washed out by warm water at second signal. Upper volume pulse record from digital artery, middle volume pulse record from pad of chilled finger; lower record from control finger of opposite hand. Time in intervals of five seconds. December.

agreement with the previously reported results of other stimuli. When the fall in finger temperature is greater (fig. 2—in this case from 33°C. to 20°C.), the constriction in the pad is more intense and more prolonged and a moderate

constriction appears in the digital artery. The constriction in the latter does not appear at once as is true for the reflex vasoconstriction in the pad, but develops slowly as the finger temperature falls. The gradual character of the constriction of the digital artery would seem to negate its origin in vasomotor reflexes. The digital artery record also shows slow "tonus" waves which are more or less synchronous with the waves in finger temperature. These waves may appear when the pad vessels remain constricted and the finger temperature is moderately below normal levels. Although the evidence is inconclusive, we are inclined to believe that these waves do not have a vasomotor origin, but that they are due to the direct effects of finger temperature on the digital artery.

Chilling with chopped ice (fig. 3) causes a more rapid and a greater fall in finger temperature and induces more marked circulatory reactions: an immediate and profound constriction of the pad vessels (and also those of the opposite hand) and a progressive but fairly rapid constriction of the digital artery. The more rapid constriction of the latter in this instance is probably related to the greater rate of fall in finger temperature with this type of cooling. The extent of the constriction is also usually greater with more complete cooling.

Although the intensity of the sensation of cold and of cold pain depends on the degree of cooling, it seems improbable that vasomotor reflexes elicited by the cold are responsible for the constriction of the digital artery. Thus, in figure 3, the constriction in the control finger is almost maximal before there has been any appreciable reduction in the amplitude of the digital pulse. Further, the gradual character of the constriction in contrast to the abruptness of the pad constriction argues against its vasomotor origin. This point is supported also by the observation that suitable chilling may induce an intense prolonged constriction in the pad without measurable effect on the digital artery pulses (fig. 4). It is probable that, in these instances, the cooling of the digital artery is inadequate to induce its constriction, and yet, that the cold sensation is sufficiently intense to cause a prolonged discharge to the pad vessels.

The reactive dilatation which occurs sooner or later during the continued application of cold is limited to the pad vessels (figs. 3 and 4). As this dilatation warms the finger, it results also in the relaxation of the digital artery. The two dilatations, the one in the pad, the other in the digital artery, may occur so nearly simultaneously that the dependence of the latter on the rise in temperature in the finger produced by the reactive dilatation in the pad may be obscured.

Occasionally, the digital artery may dilate before the pad vessels relax. It is uncertain whether this is to be considered a reactive dilatation or not. Such cases may show a high vasomotor tone in the pad during the entire observation so that the dilatation of the digital artery cannot be due to disappearance of vasomotor tone but must be due to other factors of which the finger temperature is very likely the most important.

**DISCUSSION.** These experiments show:

First, the digital artery does not participate in the initial constriction which is elicited in the pad vessels by the application of cold to the finger. This constriction has been shown previously to be due to vasoconstrictor reflexes, the

constriction possibly being augmented by the direct effects of cold on the pad vessels (7). Failure of the digital artery to constrict at this moment is in line with previously reported data showing the usual absence of vasoconstrictor reflexes in the digital artery (2). Since the vasoconstrictor discharge to the finger may be intense and prolonged, its failure to reach the digital artery implies either that this vessel does not have a vasoconstrictor supply or that the vasoconstrictor discharge is selective, reaching only the minute arteries and arterioles. Anatomical information refutes the first alternative, but it is worth emphasizing that we have not seen a convincing illustration of a vasoconstrictor reflex in the digital artery. We are inclined, however, to interpret these experiments in terms of a selective vasoconstrictor discharge.

Second, the digital artery is constricted by the direct effects of cold on the vessel. This is probably also true of the pad vessels but the effects here are obscured by the vasoconstrictor reflexes which are acting simultaneously. The extent of the constriction of the digital artery seems to be roughly proportional to the fall in finger temperature. Exact proportionality cannot be inferred from measurements of the temperature of the skin surface.

Third, the reactive dilatation which appears during the application of cold is probably limited to the pad vessels. Apparent participation of the digital artery seems to be due to the rise in temperature which results from the reactive dilatation. This indicates that the mechanism of the phenomenon is located in the minute vessels. Either the liberation of a dilator substance acting through an axone reflex on the minute arteries and arterioles as suggested by Lewis (9) or a protective dilatation of arterio-venous anastomoses as suggested by Grant (6) would be compatible with our data. It has not yet been possible to separate the relative participation of small arteries, arterioles, and arterio-venous anastomoses in the reactive dilatation in the finger. However, it seems improbable that this reaction is limited to the arterio-venous anastomoses since the amplitudes of the pad pulses at the height of the reactive dilatation would imply a wide dilatation also of the small arteries and arterioles of the pad.

It is interesting to speculate on the significance of the subject's sensations during the reactive dilatation. The throbbing is experienced with the opening of the pad vessels. It is definitely not correlated with dilatation of the digital artery. It is therefore produced by the minute vessels and may be experienced before the reactive dilatation has resulted in a pad pulse equal in size to the control level. As this reaction increases and the pad pulses become still larger, throbbing tends to disappear. Does this mean that throbbing results from the pulse entering channels which are usually closed, e.g., the arterio-venous anastomoses?

B. *The effects of cold on the propagation of the pulse in the finger arteries.* During the experiments on the effects of cold on the finger's arterial circulation, we observed that, in a few apparently normal subjects, the usually peaked form of the pad pulse may be altered to the plateau type during the reactive dilatation. This change occurred at the time when the blood flow was increasing rapidly. The similarity in the form of the pad pulse during the reactive dilatation to that

observed in resting hypertensive patients (10), suggested that we had inadvertently produced by the action of local cold in normal subjects the same changes in the arterial dynamics of the finger as exist in the hypertensive patient. The selective character of the effects of cold on the large and small arteries of the finger increased the probability of this suggestion.

**METHOD.** Analysis of the wave forms of the volume pulses of the finger pad was effected by shunting high frequency galvanometers across the recording millimeters when the previously recorded experiments on cold were performed (7) and then recording the pulses at suitable moments on a separate high-speed photo-kymograph. Recording of the wave forms was so selected at the time of the experiment that representative samples of the volume pulse waves were secured during dilatation and constriction in the control, experimental, and recovery periods.

Analysis gave attention to the form of the wave and the crest times, following the procedure as previously reported (10). The crest times of the pad volume pulses depend on the time required for the minute pad vessels to reach peak filling during an individual pulse cycle. The crest times are therefore affected by the relative constriction of the digital artery and of the minute arteries of the pad. Selective constriction of the latter may be expected to shorten the crest time of the pad pulse. Selective dilatation of the pad vessels in the reactive dilatation due to cold may, on the other hand, increase considerably the crest time due to the larger capacity of these vessels and the possibly slower arrival of the pulse wave through the partially constricted digital artery. It will be seen that these effects on the crest times are not measurable unless the changes in the lumens of the digital artery and of the pad vessels are considerable. Moderate changes as in mild vasomotor reflexes do not measurably influence the propagation of the pulse in the finger arteries (10).

**RESULTS.** In the usual experiment (fig. 5 and table 1) the effects of cold on the propagation of the pulse were minor; there was little change in the form of the pad pulse. During the onset of the initial constriction (with the application of cold), crest times were shortened in both control and experimental fingers but more so in the latter in which the constriction was also greater. As constriction continued, the crest times were lengthened towards the values of the control period. The dicrotic notch was placed higher on the catacrotic limb during the constriction. As the reactive dilatation began, the crest times were lengthened slightly, returning to normal values when the vessels were well dilated. The dicrotic notch was usually placed higher at the beginning of the reactive dilatation, falling again as the latter developed. The pulse waves had the usual peaked form during the constriction but tended to be very slightly rounded during the reactive dilatation. In general, then, the effects of the constriction to cold and of the subsequent dilatation, on the form of the finger pulses, were very moderate.

However, in several apparently normal subjects, there occurred reversible changes in the wave forms which resembled those seen in resting hypertensive and arteriosclerotic subjects (10). We present two examples. In the experiment



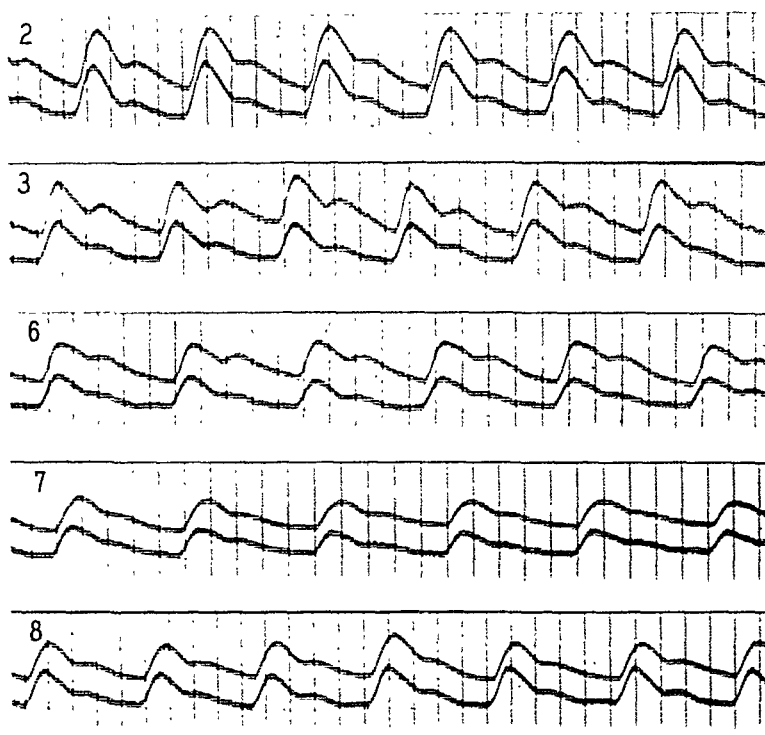


Fig. 5. Records of volume pulses by high frequency galvanometers taken at vertical signals in figure 1 of preceding paper (7). Records 2, 3, 6, 7, 8 were taken at the moments numbered correspondingly in that figure. Records 3 and 6 of the chilled finger's pad pulses were taken with increased amplification. Upper wave, chilled finger; lower wave, control finger. Time: 0.15 sec.

TABLE 1

*Crest times and position of dicrotic notch on finger pad volume pulses during local application of cold to the finger*

RECORD	CREST TIMES IN SECONDS		ELEVATION OF DICROTIC NOTCH IN % OF WAVE		PERIOD OF EXPERIMENT
	Control finger	Chilled finger	Control finger	Chilled finger	
2	0.12	0.13	41	50	Control
3	0.11	0.12	47	65	Control—during slight constriction
4	0.08	0.06	40	74	Initial constriction to cold
5	0.13	0.14	40	68	Early in reaction
6	0.13	0.13	45	61	Later in reaction
7	0.09	0.07	53	57	During marked constriction. Cold
8	0.10	0.11	52	61	Later in constriction. Cold

Record numbers correspond to those in figure 5 of this paper and in figure 1 of the preceding paper (7).

of figure 6, we note no unusual effects on the time relations. But the tendency towards a plateau in the pulse of the chilled finger is exhibited during the reactive dilatation when the pulse of the control finger is peaked and when the volume

pulse amplitudes are the same in the two fingers (records 7, 13, 16 of fig. 6). The plateau is due to elevation of the dicrotic notch and the "zwischen-schlag". The latter is a small wave between the crest of the pulse and the dicrotic notch. There is a faint suggestion of it in the control record 2. It is very obvious in the pulses of the control and chilled fingers in records 13, 16 and 17. It is the crest of the wave of the chilled finger in the first, second, third, and fifth pulses of rec-

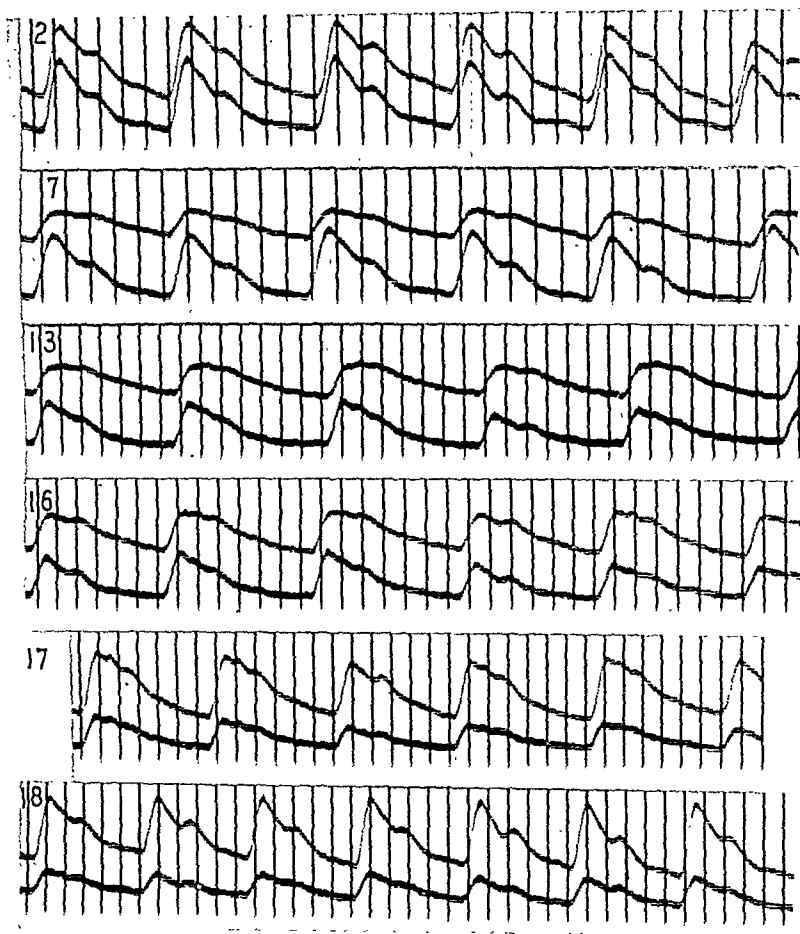


Fig. 6. Records of volume pulses of same subject as in figure 4 of preceding paper (7), but from a different experiment. Record 2, during control period. Record 7, during well developed reactive dilatation (control finger temperature  $35^{\circ}\text{C}.$ ; chilled finger temperature  $16^{\circ}\text{C}.$ ). Record 13, during the reactive dilatation (finger temperatures  $34.6^{\circ}\text{C}.$  and  $14^{\circ}\text{C}.$ ). Records 16, 17, 18, during early part of recovery; record 16, 2 minutes after application of cold was stopped (temperatures  $35^{\circ}\text{C}.$  and  $15^{\circ}\text{C}.$ ); record 17, 2 minutes later (temperatures  $35^{\circ}\text{C}.$  and  $28^{\circ}\text{C}.$ ); record 18, 6 minutes later (temperatures  $35^{\circ}\text{C}.$  and  $34^{\circ}\text{C}.$ ). Upper wave: chilled finger; lower wave; control finger. Time 0.15 sec.

ord 13. If the crest time were considered as the time to the "zwischen-schlag", this would give a considerable increase in the value as was found to be the case in hypertension and arteriosclerosis (10). It was suggested then that ascent of the "zwischen-schlag" was responsible for the increase in crest time. We have here an example of the experimental reversible production of a similar effect in a subject with apparently normal arteries.

The experiment of figure 7 was done somewhat differently on an older subject in good health. The finger was immersed in chopped ice and the records taken after the finger had been withdrawn from the ice and while it was exposed to room air. This procedure is most effective in securing marked constriction of the digital artery. Record 2 was obtained immediately on withdrawal from the ice

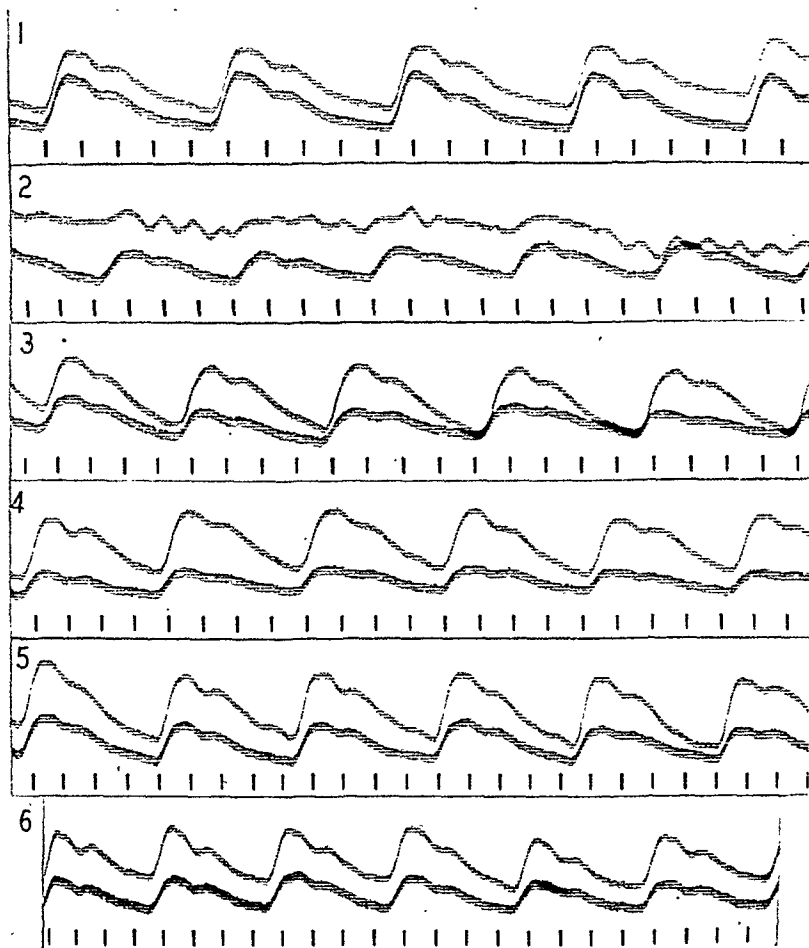


Fig. 7. Effects of ice-water on finger volume pulses. Subject: male 42 years old. Normal blood pressure. Record 1, during control period. Record 2, immediately following immersion in ice water for 2 minutes—finger cold, aching, burning. Record 3, immediately following immersion for 6 minutes. Finger warm, throbbing. Record 4, 1 minute later. Record 5, 2 minutes after record 3. Record 6, 4 minutes after record 3. Upper wave: chilled (R2). Lower wave: control finger (R4). Time 0.2 sec.

while the finger was constricted. Records 3 to 6 were taken in another run during the reactive dilatation which began while the finger was still in the ice. The control record, number one, shows a peaked wave with a slight tendency towards a plateau due to high location of the dicrotic notch and the “zwischen-schlag”—the latter is barely discernible. The crest times are normal in this control record. During the reactive dilatation, the “zwischen-schlag” ascends

to become the crest of the wave. This increases the crest time to the values found in hypertensive and arteriosclerotic subjects.

INTERPRETATION. Theoretical considerations suggest the following possibilities in the effects of the constriction to local cold and of the subsequent reactive dilatation on the propagation and form of volume pulses in the finger:

1. Constriction of the pad arteries without much change in the digital artery trunks should increase digital artery pressures somewhat with resultant slightly faster propagation of the pulse and also decrease the time required for the pad vessels to reach peak filling during the pulse cycle. The crest times should therefore be shortened. This happens regularly during the early part of the initial constriction to cold. We have shown above that the digital artery trunks do not participate significantly in the constriction at this time. This fact is in agreement with the shortening of the pad crest time during the initial constriction to cold.

2. Constriction of the digital artery trunks as well as of the pad arteries later during the application of cold would tend to offset the effects of constriction in the pad arteries on the crest times. These should increase slightly, as they do, a little later in the initial constriction, due to slower propagation of the pulse.

3. Continued considerable constriction of the digital artery trunks with progressive relaxation of the minute pad arteries (possibly the arterio-venous anastomoses and arterio-venous shunts) would slow the propagation of the pulse, increase the time for peak filling of the minute vessels during the pulse cycle, and so increase the crest time. This is the case early during the reactive dilatation which is limited to the pad vessels and which does not affect the digital artery trunk until the rise in finger temperature causes relaxation of this vessel. Quantitative differences between individuals may depend on the relative effects of the degree of constriction of the digital trunks and of the pad arteries on the propagation of the pulse wave. This situation is analogous to that described by Grant (4) in the rabbit's ear in which the main artery trunks of the chilled ear remained partly constricted until the opening of the arterio-venous anastomoses permitted a larger flow of blood with a rise in the ear temperature.

4. When dilatation of the minute pad vessels (arterio-venous anastomoses, etc.) is followed by dilatation of the digital artery trunk later in the reactive dilatation, it should result in a decrease of the crest time to normal values. This is the case in the later stages of the reactive dilatation.

This interpretation of the changes in the form and crest times of the pad pulses during the application of cold is in line with the direct demonstration (above) of the selective effects of cold on the finger's arterial circulation. It may be noted, however, that this theoretical interpretation was formulated from the pad pulse records before the experiments on the digital artery were carried out.

Evaluation of the meaning of the changes in wave form illustrated in figures 6 and 7 is a more difficult problem. Ordinarily, a plateau form of the peripheral arterial pulse is associated with an increased peripheral resistance and an altered elasticity of the vessels. In these two experiments, the plateau form appears also at the time when the vessels are dilated, when the flow is high and the finger is

throbbing. At the same time, the pulse in the neighbouring control finger may be peaked (fig. 6), so that the plateau in the pulse in the chilled finger must be due principally to the local arterial dynamics in the chilled finger. The effects of selective changes in tone of the digital artery trunks, of the pad arteries and arterioles, and of the arterio-venous anastomoses, on the reflection and damping of pulse waves, are pertinent to interpretation but must be considered a purely speculative matter. However since the plateau form disappears when the reactive dilatation is well advanced (record 18 of fig. 6) and the digital artery trunk is probably dilated, it seems possible that the plateau form develops when the arterio-venous anastomoses and other small arteries are widely dilated and the larger arteries are still constricted. As the finger warms, the arterio-venous anastomoses probably close, the larger arteries are dilated again and a rapid flow occurs through the usual channels. This reestablishes the usual arterial dynamics in the finger. The changes illustrated in figure 6 fit this concept which is supported by the experiments described above as well as by the direct observations of Grant on the rabbit's ear (4).

We may then ask, why does not plateau formation appear in the usual experiment? In a sense, it does, as the dicrotic notch is elevated (higher than in the control) in nearly all instances during the early part of the reactive dilatation. Differences in the effects of the latter on the wave form of normal subjects may then be due to quantitative differences in the degree of participation of the components of the finger's arterial system and are not necessarily due to qualitative differences in behavior.

The striking similarity in the effects of hypertension and of cold in some normal subjects on the propagation of the pulse in the finger arteries poses some interesting problems. It has been argued from measurements of brachial-digital pressure gradients that the increase in resistance in hypertension is located in the small arteries and arterioles of the finger (11). If this is all that happened, the crest times of the pad pulses should be shortened, as is the effect of pad constriction in the normal subject. But the reverse has been observed without exception in every case of hypertension studied (10, and unpublished data). If there were an increase in the resistance in the digital artery and of other large hand arteries in hypertension (which is denied by the brachial-digital pressure gradients) this would lengthen the pad crest time, but the constriction of the digital artery in the normal subject does not increase the pad crest time to the values observed in hypertension. This does not necessarily eliminate the digital artery and other large hand arteries from modifying the pulse in its propagation into the minute vessels of the finger pad. This possibility is being studied.

There remains the possibility in hypertensive cases that blood is shunted along such channels as the arterio-venous anastomoses, as may also be the case during the reactive dilatation in normal subjects. The extent to which these vessels can affect the finger pulses and blood flow is unknown. However, if their opening occurs at a time when the small arteries and arterioles are constricted (as in the hypertensive patient and during cold in the normal subject)

and if their dilatation can restore blood flow in the finger, the form of the pad volume pulses may well differ from the normal. It is therefore extremely interesting that, in some normal subjects, the reactive dilatation to cold (when the arterio-venous anastomoses presumably open) results in a pad volume pulse which is similar to that seen in hypertensive patients.

#### SUMMARY

A. The selective effects of local cold on the terminal pad vessels and the digital artery of the chilled finger were demonstrated by means of photoelectric plethysmographs.

The digital artery does not participate in the vasoconstrictor reflexes elicited by the cold. Its later constriction during the continued application of cold appears to be due to the direct effects of the fall in temperature on the artery.

The reactive dilatation which appears during the application of cold is limited to the minute pad vessels and does not involve the digital artery until the resultant rise in finger temperature permits relaxation of this artery.

B. The effects of these reactions on the propagation of the pulse in the finger's arterial system were studied by recording the pad pulses with high frequency galvanometers.

In the usual experiment, the time relations and form of the pad pulses in the chilled finger were altered only moderately and in the direction which could be predicted from the relative participation of the pad and digital arteries in the reactions to cold.

In a few normal subjects, the reactive dilatation produced a pad pulse similar to that seen in chronic hypertension, thus suggesting that one of the factors responsible for the change in pad pulse form in hypertension may be the shunting of blood through direct arterio-venous communications.

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# THE ABSENCE OF VASOCONSTRICTOR REFLEXES IN THE FOREHEAD CIRCULATION. EFFECTS OF COLD<sup>1</sup>

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Plethysmographic data were presented in earlier publications (1, 2, 3) to illustrate the selective character of vasomotor activity in the skin. These plethysmograms indicated the absence of vasoconstrictor reflexes in head skin (with the exception of the ear). It has been suggested that these results "may be due rather to relative vasomotor inactivity of certain vessels than to an independence of rhythmic activity" (4).

It is possible that vasoconstrictor fibers do not reach the forehead skin. There are many observations which so suggest: the constancy of the forehead temperature and its failure to fall during prolonged constriction of the fingers and toes; the throbbing of the temporal artery during excitement; the frequent increase in forehead volume pulses during vasoconstrictor discharges (1); the relative constancy of the blood supply of head skin during massive disturbances of the circulation by drugs (5); the failure of unilateral cervical sympathectomy to increase the temperature of the forehead skin on that side (6)—the effects are also very small on the cheek, upper lip, nose and chin.

The present communication reports data which extend this list and further deny the existence of vasoconstrictor reflexes in the forehead skin. The reactions of the minute arteries in the forehead skin were studied by recording their volume pulses with the photoelectric technique which has been described elsewhere (7).

**RESULTS.** Spontaneous arterial constrictions which are seen so commonly in plethysmograms of the extremities and which are generally considered as due to vasomotor activity, have not been observed in the forehead skin. A striking illustration is provided in figure 1 obtained from a normal male while he rested, slept, and awakened. The frequent vasomotor discharges to the finger are wholly absent in the forehead. Reduction in finger flow (third record), probably due to increased vasomotor tone, occurred without evident change in the forehead circulation.

Startle due to loud unexpected noises, immersion of the hand in ice water, or a deep breath, are known to elicit vasoconstrictor reflexes which not only affect both the upper and lower extremity, but also are often sufficiently extensive to increase the arterial blood pressure. If these stimuli are effective, the finger's

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vessels are always constricted in the normal subject. At times, the vasoconstrictor reflexes so elicited may include the vascular areas in the calf and forearm (8), but the constrictor effects here are weak compared to those in the hand and foot. We have seen no instance of arterial constriction in the forehead in response to these stimuli, irrespective of how intense the effects may have been in the finger. Figure 2 illustrates. The stimuli of a loud noise, of immersion of the opposite hand in ice water and of a deep breath were supplied in succession. The effect of awakening the sleeping subject by the observers' cough is also

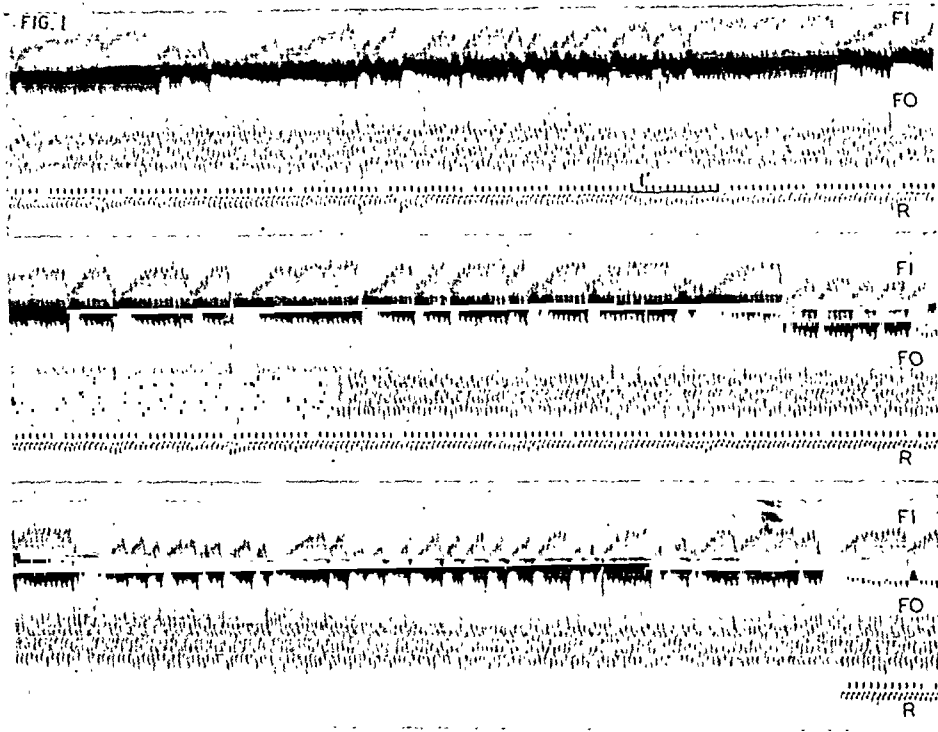


Fig. 1. Effects of spontaneous vasomotor activity on the finger and forehead vessels in the resting subject who was asleep and snoring during most of the record, which is continuous. Upper volume pulse record from finger, lower from forehead. Respiration. Time: 5 seconds. Records of temperatures of finger and forehead taken by thermocouples do not show in the reduction of the figure but show no large changes in the original. Room temperature varied between 23°C. and 25°C.

shown. Decisive constriction occurred in the finger in each case. If there had been a weak constriction in the forehead skin, there would have been at least a slight decrease in the volume pulse here. But there is no indication whatever in these records of a vasoconstrictor action on the forehead arteries.

An effective method of eliciting constriction in cutaneous vessels is the local application of cold. Effective chilling of the forehead skin in such a manner as to permit simultaneous and continuous recording of the volume pulses of the chilled skin proved to be fairly difficult. We tried various applicators made of rubber dam through which cold water could be circulated. These were large enough to cover most of the forehead. A hole cut in the center permitted the



plethysmograph to reach the small skin area which was surrounded by skin in contact with the cold surface of the applicator. Dissatisfied with the small fall in temperature so induced in the skin directly beneath the plethysmograph,

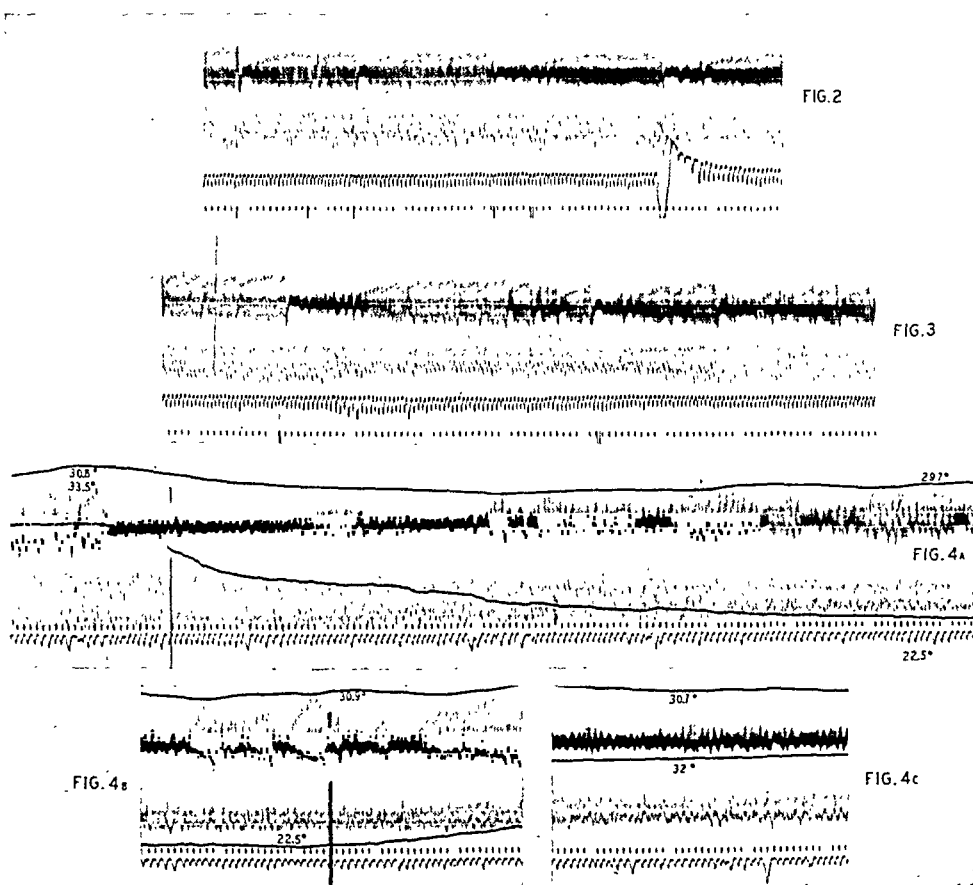


Fig. 2. Absence of vasoconstrictor reflexes in forehead skin. At 1, the sleeping subject was awakened by the observer's cough. At 2, a loud noise. At 3, immersion of the opposite hand in ice water. At 4, a deep breath. Upper volume pulse record from finger pad, lower from forehead. Respiration. Time: 5 sec.

Fig. 3. Effects of moderate cold applied to the forehead by means of cold water circulated through a hollow rubber applicator which was in contact with the forehead. The cold was applied between the vertical signals. Upper volume pulse record from the finger pad, lower from the forehead. Respiration. Time: 5 sec.

Fig. 4. Effects of more prolonged application of more intense cold than that applied in figure 3. The cold was applied at the break in the forehead temperature curve. Recovery of the forehead circulation was incomplete at the end of the observational period. No pain was experienced. The time intervals between the sections of the figure are: between sections 1 and 2, 12 minutes; between sections 2 and 3, 21.5 minutes. The records from above, downwards: finger pad temperature, finger pad volume pulse, forehead temperature, forehead volume pulse, time (5 sec.), respiration.

we constructed an applicator of segments of copper sheeting which were soldered to short pieces of copper tubing. The latter were connected closely together by rubber tubing. This arrangement provided an excellent broad comfortable

contact with the forehead skin. A gap in its middle provided for the application of the plethysmograph, as with the rubber applicator. Despite the excellent contact with the skin and the high thermal conductivity of copper, this device was not much more successful than the rubber applicator in lowering the temperature of the skin either directly under it or under the plethysmograph. The

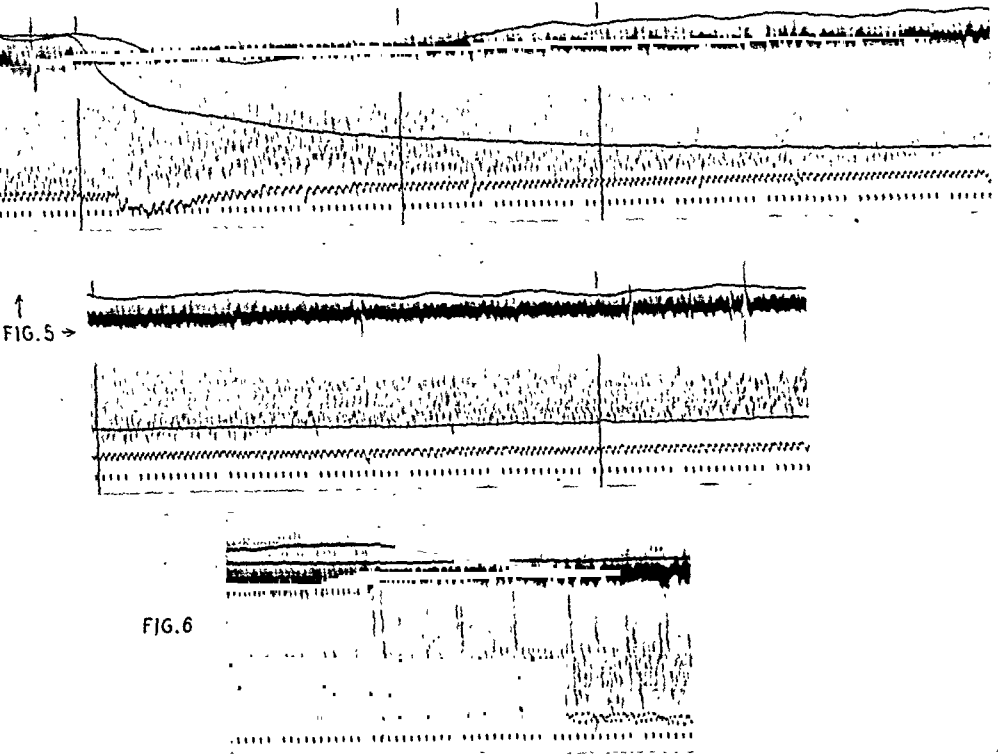


Fig. 5. Relation of the effects of cold on the forehead circulation to the sensations experienced. The cold was applied to the forehead by means of a special copper applicator, at the first vertical signal. At the second signal, there was intense pain which was localized under the applicator. At the third signal, the pain was somewhat less intense. At the fourth signal, the pain was described as moderate. At the fifth signal, the pain has disappeared. Records from above, downwards: finger pad and forehead temperatures, finger pad volume pulse, forehead volume pulse, respiration, time (5 sec.).

Fig. 6. Temporary increase in the forehead volume pulse on the application of cold to the forehead. "Quite intense pain for the first minute or so" was reported by the subject. The cold was applied just preceding the constriction in the finger. The artifacts in the forehead record were due to frequent swallowing movements. Upper volume pulse record from the finger pad; lower from the forehead. Respiration. Time: 5 sec.

cold applicator and the plethysmograph were suspended from a plaster of Paris cast of the subject's cranium as illustrated elsewhere (9). This device is comfortable and permits prolonged observations.

On starting the flow of cold water (chilled to 4–8°C.) through the applicator, constriction occurred at once in the control finger but not immediately in the forehead skin which was being cooled (figs. 3–6). The intensity and duration of the constriction in the finger varied with subjects; there was no obvious correla-

tion with the sensations. Constriction appeared gradually in the forehead skin as its temperature fell. Cooling took place rather slowly and its action on the forehead vessels required periods as long as twenty minutes to attain a maximal decrease in the volume pulse. The cooling was insufficient in all instances to secure complete constriction. The decrease in pulse amplitude varied in the individual experiments between 32 and 90 per cent of the control value. In no instance was there any indication of a vasoconstrictor reflex in the forehead skin, although in many trials the reduced volume pulses and the increased frequency of "spontaneous waves" in the finger during the cold application showed that the cold stimulus continued to be effective in eliciting vasoconstrictor reflexes.

The absence of the initial constriction to cold in the forehead skin is comparable to those rare cases described in a previous paper (10), in which chilling of the finger or toe results in *gradual* constriction when vasoconstrictor reflexes are *not* elicited by the cold. Also, the sympathectomized toe responds to cold as does the forehead skin (10). These analogies in responses strongly support our thesis that vasoconstrictor reflexes are wholly absent from the forehead skin.

In a few experiments (figs. 5 and 6), the forehead volume pulse was considerably increased during the cold application at the time of sharp constriction in the finger (fig. 6) or when pain in the forehead was experienced (fig. 5). This effect may have been due to an increase in pulse pressure or to a vasodilator reflex elicited in the forehead by the pain. There is evidence that vasodilator fibers reach the forehead by way of the cervical sympathetic (6). Thus, neither emotional flushing nor the flushing due to over-heating occur on the denervated side of the face and forehead after unilateral cervical sympathectomy (6).

The increase in amplitude of the forehead volume pulses (fig. 5) with the onset of pain, when constriction of the forehead vessels due to cold had already occurred, recalls the correlation between the amplitude of the temporal artery pulsations and the presence or absence of headache (11). However, none of the subjects confused the pain from the local cold application with headache. The pain was somewhat similar to a sharply localized headache but contrasted notably with the intense frontal ache produced by strong winds on a bitter cold day. The difference seemed to be primarily one of intensity and may well have been due to failure to cool adequately. Some subjects experienced only a sensation of cold.

The reactive dilatation which is so characteristic of the finger's reactions to cold was not observed in the forehead skin. This may have been due to inadequate cooling as we seldom succeeded in chilling to the point where the constriction was sufficient to eliminate the volume pulse. The lowest temperature reached in these experiments was 21°C. which is above that at which the reactive dilatation is usually induced in the finger. It is also possible that the arterio-venous anastomoses are too few in number in the forehead skin for a reactive dilatation to occur. Information is lacking on this point.

Recovery of the forehead circulation and of the forehead temperature after ending the cold applications proceeded slowly, thus contrasting sharply with the recovery in the finger (10) where the rise in temperature and the arterial dilata-

tion occurred rapidly. The slowness of recovery in the forehead may have been related to the absence of any indication of a reactive dilatation there. It may also have been affected by the thermal capacity of the cold applicator. Yet, recovery in the finger was also subject to the same delaying influence but proceeded swiftly.

#### SUMMARY

The absence of vasoconstrictor reflexes in the forehead is demonstrated by the following evidence:

1. The spontaneous rhythmic constrictions of vasomotor origin in the finger of the resting subject are absent from the forehead.

2. The vasoconstrictor reflexes elicited by startle, awakening, a deep breath, immersion of the hand in ice water or local cold to the forehead are wholly absent in the latter.

3. The vascular reactions of the forehead skin to local cold are like those of a sympathectomized digit. Constriction is gradual as the temperature of the skin falls and seems to be due to the direct effects of cold on the vessels.

The reactive dilatation to cold which occurs in the fingers, was not observed in the forehead skin.

Occasionally, there was indication of a vasodilator reflex in the forehead skin at the time of a powerful constriction in the finger.

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## THE EFFECTS OF VERATRINE UPON THE SUPERIOR CERVICAL GANGLION

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Injections of veratrine result in striking changes in many of the properties of axons (see for references Acheson and Rosenblueth, 1941). The present study was carried out with the purpose of comparing some properties of the superior cervical ganglion with those of axons. The assumption underlying this comparison was that if the properties of the two structures are similar the action of veratrine should likewise be similar, at least qualitatively.

**METHOD.** The animals used were cats, under dial anesthesia (Ciba, 0.7 to 0.75 cc. per kgm., intraperitoneally). In a series of observations the preganglionic fibers in the cervical sympathetic were stimulated and the electric responses of the ganglion were recorded from one lead on this structure to another on the crushed postganglionic fibers. In other animals the responses of the nictitating membrane were recorded upon stimulation of either the pre- or the postganglionic fibers. In still other cases both the mechanical responses of the membrane and the ganglionic electrograms (diphasic) were simultaneously registered when the preganglionic trunk was activated. Finally, in some instances the preganglionic electric responses were observed in addition to those of the ganglion, postganglionic fibers, or nictitating membrane.

The contractions of the nictitating membrane were recorded isotonically on a kymograph. The eyeball was enucleated to minimize disturbing movements due to the periocular striated musculature.

The electric responses were photographed from a cathode-ray oscillograph after amplification. Direct-coupled amplification was used in some cases to avoid distortion of slow potential changes. In other cases a capacity-coupled amplifier was employed.

Both for stimulation and for recording, the electrodes were chlorided silver wires, insulated by rubber or by glass from surrounding tissues. From 1 to 3 pairs of these electrodes were placed as follows: *a*, on the post-ganglionic fibers or the ganglion; *b*, on the preganglionic trunk, near the ganglion; *c*, on the preganglionic trunk at the base of the neck. The preganglionic fibers were invariably cut as close to the thorax as possible. Whenever electrodes were placed

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on the ganglion or postganglionic fibers, the neighboring X, XI and XIIth nerves were cut at the base of the skull and a stretch of 1 to 2 cm. was excised.

The stimuli were condenser discharges through a thyatron. They were usually rendered diphasic by a transformer before application to the nerve.

Veratrine was injected intra-arterially. The external carotid was ligated. The injections were made retrogradely into the cannulated lingual artery. The intra-arterial injections ensured a prompt delivery of the drug to the ganglion

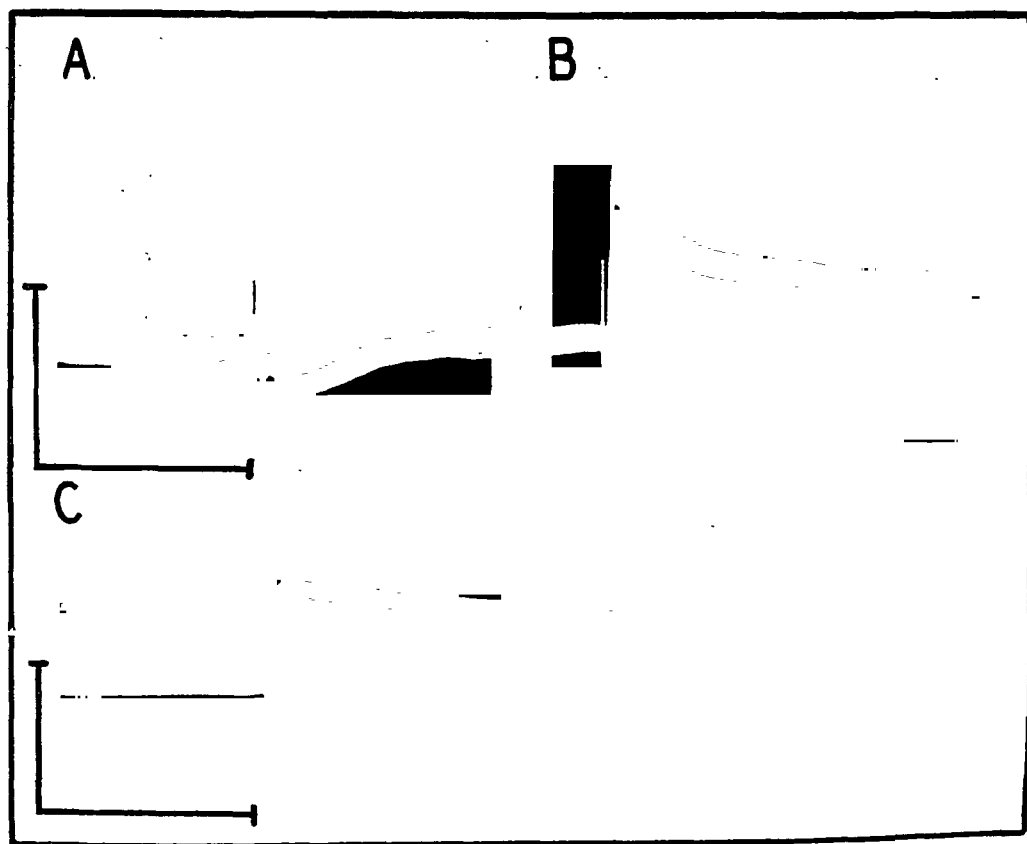


Fig. 1. Responses of the superior cervical ganglion to stimulation of the preganglionic fibers by single maximal shocks. Direct coupled amplifier. In this and other ganglionic electrograms upward excursions denote negativity of the ganglion with respect to the postganglionic fibers.

A, before, and B, after injection of veratrine (0.2 mgm.). Voltage calibration: 0.1 mv.; time calibration: 0.5 sec.

C. In another animal. After veratrine (1 mgm.). Voltage calibration: 1 mv.; time calibration: 1 sec.

and minimized undesirable general effects. The ligation of the external carotid decreased the amount of veratrine which reached the nictitating membrane.

**RESULTS.** A. *Electric responses to single shock stimulation.* Under this heading will be considered the action of veratrine on the responses to preganglionic stimulation by single maximal shocks, recorded from one lead on the surface of the ganglion to another in contact with a crushed portion of the postganglionic fibers.

The demarcation potential was measured by the counter e.m.f. used to balance the potential differences between the electrodes for recording with the direct-coupled amplifier. Its value at the beginning of the experiments was about 10 to 20 mv. Veratrine (0.2 to 2 mgm.) caused a decrease of about 2 to 10 per cent.

The amplitude of the ganglionic spike potentials was increased by moderate doses of veratrine (0.2 to 0.5 mgm.). The measurements were made mainly on

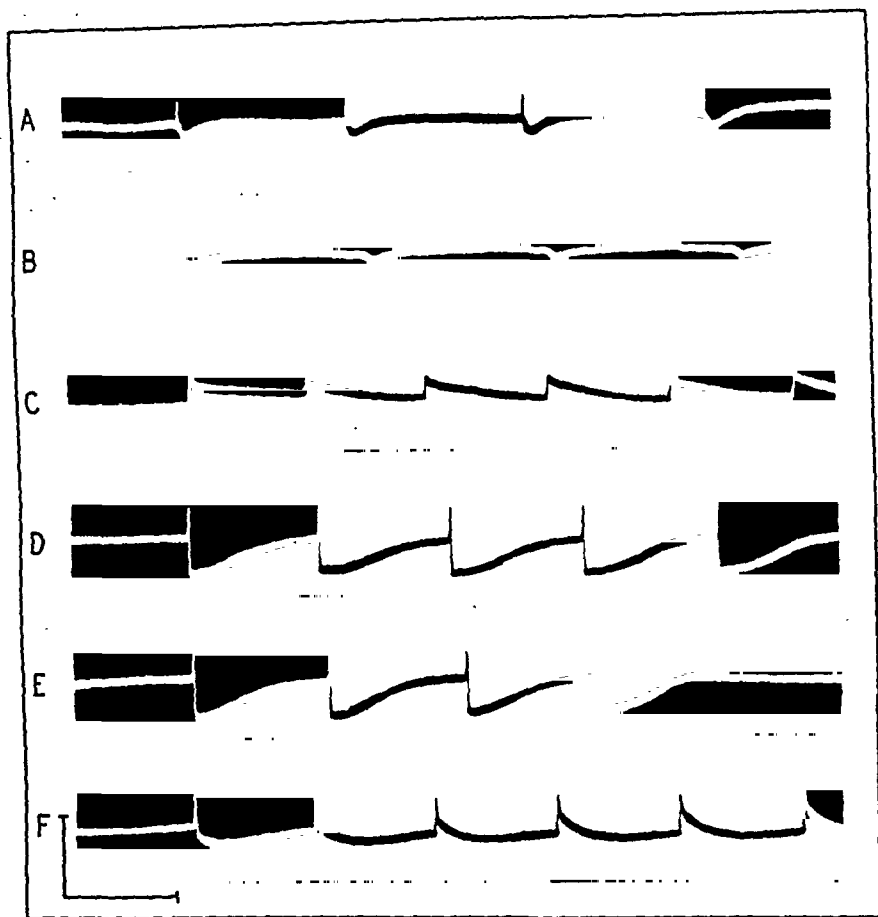


Fig. 2. Increase of the positive after-potential after veratrine. Direct-coupled amplifier. Monophasic records from the ganglion. Maximal stimulation of the preganglionic fibers at the frequency shown by the responses. Voltage calibration: 1 mv., time calibration: 1 sec.

A, before veratrine. B, 1 min. after injection of veratrine (1 mgm.). The record corresponds mainly to a positive after-potential. C, 20 min. after veratrine. D, 100 min. after veratrine. The spike and the positive after-potential are larger than normal. E, 140 min. after veratrine. The spike is normal but the positive after-potential remains large. F, 5 min. after injection of veratrine (0.2 mgm.), administered immediately after E. The spike is increased; the apparent decrease of the positive after-potential is due to an increase of the negative after-potential, which is seen to grow with the successive shocks.

the spikes corresponding to the  $M_2$  group of elements (Bishop and Heinbecker, 1932), which contribute the largest spike component recorded from the ganglia of cats. Figure 1 (A and B) illustrates a typical observation. In an extreme case the spike potential doubled in amplitude after veratrine. Large doses of veratrine (1 to 3 mgm.) caused a decrease of the spikes. This decrease may



have been due to block of some elements (see below), rather than to a decrease of the spike magnitude per element.

Whether the spikes were greater or smaller than normal after veratrine their rate of development was slower than before administration of the drug—i.e., the slope of the ascending branch of the spike became less steep and the time to reach the peak became longer after injections of the drug. The total duration of the spike could not be judged because of the presence of the negative after-potential.

The negative after-potential of the ganglion (N wave of Eccles, 1935b) was markedly increased by veratrine (figs. 1, 2, 5, 7 and 8). This potential is usually small in normal ganglia, and is promptly followed by a prominent positive after-potential (P wave of Eccles, fig. 1A). With progressively increasing doses of veratrine the residual negativity increased gradually at the expense of the positivity and could entirely mask the latter. After large doses, the peak of the negative after-potential occurred usually some time after the peak of the  $M_2$  spike and could reach values as high as 130 per cent of the spike (fig. 1C). It could last over 10 sec.

The decrease of the positive after-potential after veratrine mentioned above might be interpreted as a direct action of the drug, rather than as an algebraic summation of the two after-potentials of opposite sign. This interpretation is probably erroneous, however, for veratrine causes an increase of the positive after-potential. This increase was readily seen in responses obtained some time (30 to 60 min.) after an injection of veratrine. As time proceeds the initially large negative after-potential declines and a marked residual positivity appears, which will in turn recede later. Figure 2 illustrates a typical observation.

An increase of the positive after-potential was also demonstrated by the effects of large doses of veratrine, sufficient to block all ganglionic responses. The first thing to appear when the block subsided was a very small spike and a relatively large positive wave (fig. 2B; the spike is not visible without higher amplification).

The ganglionic responses became repetitive after injections of even small doses of veratrine (e.g., 0.2 mgm.). The repetitive character of the discharge was not obvious in the monophasic records with direct-coupled amplification, since any spikes after the first volley were masked by the large residual negativity. In diphasic records with capacity-coupled amplification the repetitiveness of the responses was clear (fig. 3B). Repetitive postganglionic activity was further indicated by an increase of the responses of the nictitating membrane (fig. 4). Veratrine causes repetition in the preganglionic fibers (fig. 3A). Such multiple preganglionic discharges will elicit repetitive responses from the ganglion. It is difficult, therefore, to decide whether the drug renders the cell-bodies at the ganglion repetitive—i.e., whether a single preganglionic volley would result in multiple ganglionic discharge.

*B. Responses to repetitive stimulation.* The effects of repetition of the stimuli, at rates of 0.2 to 60 per sec., on the ganglionic responses varied with the dose of veratrine administered to a given animal. The action of a certain dose, however, varied considerably for different animals. For this reason typical effects

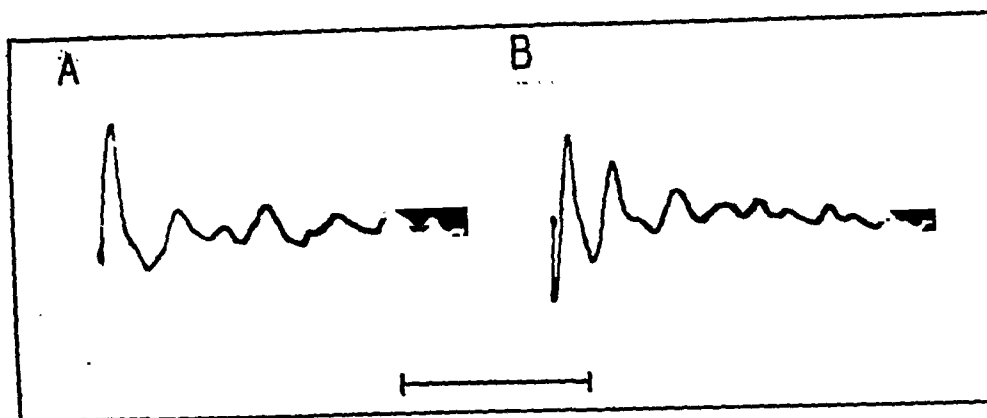


Fig. 3. Repetitive discharges of preganglionic and postganglionic fibers in response to single shock stimulation of the preganglionic nerve after veratrine (0.5 mgm.). Time calibration: 10 msec.

A, monophasic record from the preganglionic fibers. B, diphasic record from the postganglionic fibers.

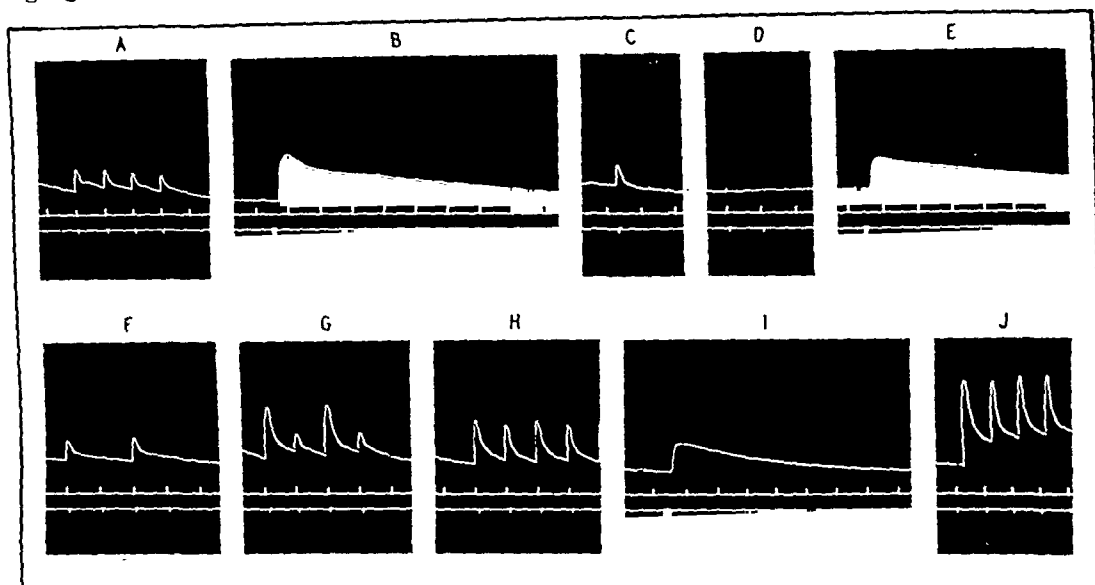


Fig. 4. Responses of the nictitating membrane. Stimulating electrodes on the preganglionic (pre.) and postganglionic (post.) sympathetic fibers. Stimulation by single maximal shocks. In this and other records from the membrane the time signal denotes 30-sec. intervals.

A, stimulation: post., pre., post., pre., at signals. B, intravenous injection of adrenaline ( $10\gamma$ ). C, intra-arterial injection of veratrine (1 mgm.). D, one minute later. Stimulation: post., pre. E, intravenous injection of adrenaline ( $10\gamma$ ). F, five minutes after veratrine. Stimulation: post., pre., post., pre. G and H, as in F, 5 and 10 min. later, respectively. I, Adrenaline ( $10\gamma$ ). J, the responses of the membrane had returned to normal about 20 min. after I. Veratrine (0.2 mgm.) was then injected and this record of stimulation (post., pre., post., pre.) was taken 1 min. later.

will be described, instead of attempting to systematize the results on the basis of the doses of the drug.

Both the ganglionic spikes and the negative after-potential showed commonly a characteristic change of amplitude upon repetitive maximal stimulation at

rates of 0.2 to 10 per sec. The magnitude of the potentials first decreased sharply and later gradually increased. Figure 5A illustrates an example.

As shown by Acheson and Rosenblueth (1941) the somatic axons A of the cat exhibit similar properties after injections of veratrine. It might be surmised, therefore, that the decline followed by increase of the ganglionic responses could be due to corresponding changes at the stimulated preganglionic fibers. This possibility was tested in some animals by stimulating the preganglionic trunk close to the ganglion and recording first from the ganglion and then from the cut central end of the preganglionic fibers with a constant frequency of stimulation. Although the preganglionic nerves exhibited sometimes a slight decrease, then increase of the responses, it was much smaller in degree, and was not parallel in time course, when compared with the corresponding change of amplitude in the ganglionic records (cf. fig. 5A and B).

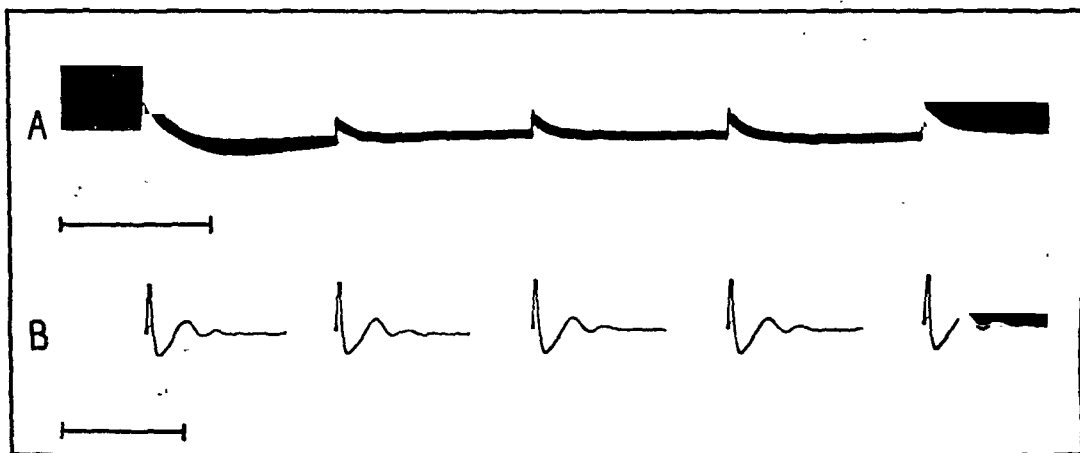


Fig. 5. Independence of the amplitude of the ganglionic spike potential and the corresponding negative after-potential. Capacity coupled amplifier.

A, ganglionic record, time calibration 1 sec. B, corresponding preganglionic responses, time calibration 50 msec.

In other animals the preganglionic fibers were stimulated near the cut central end and the record was taken from a preganglionic lead close to the ganglion to a ganglionic lead. In such records some preganglionic components could be clearly identified and separated from some of the ganglionic components. A total lack of parallelism was again seen between the changes of amplitude in these components.

In rare instances an initial decrease followed by a later increase of the amplitude of the positive after-potential in successive responses was seen after veratrine. Figure 6 illustrates the phenomenon.

An alternation of the magnitude of ganglionic responses to successive shocks of uniform frequency and maximal intensity was occasionally encountered. The frequency most favorable for the appearance of the phenomenon was about 1 to 2 per sec. Higher or lower rates did not lead to alternation.

With certain doses of veratrine and certain rates of stimulation either the negative or, more rarely, the positive after-potentials tended to increase pro-

gressively in amplitude with repetitive stimulation. Thus, in figure 2F is shown a progressive increase of the negative after-potential with successive shocks. In general, when the negative wave was large (e.g., after a large dose of veratrine) it tended to decrease with repetition; conversely, if it was relatively small (small dose of veratrine), it tended to increase with repetition of the stimuli.

The increasing effect of veratrine on the positive after-potential was obvious when, at certain intermediate frequencies (5 to 10 per sec.), greater than normal residual positivity developed; slower or faster frequencies led then to increased negativity, instead of positivity.

The transmission of nerve impulses across the ganglionic synapses could best be judged by the records of the nictitating membrane. Normally the contractions of the membrane are well sustained with frequencies of preganglionic

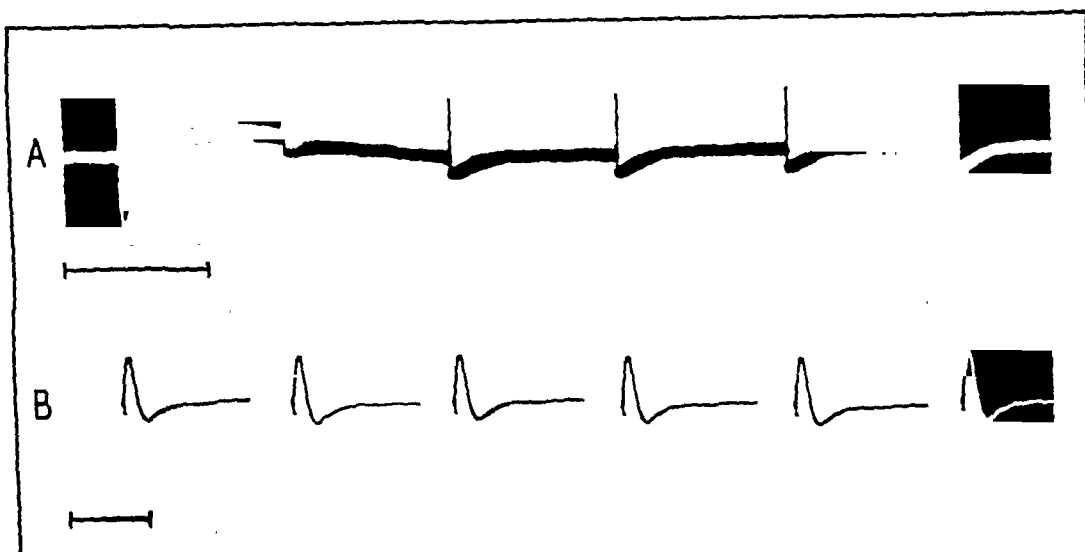


Fig. 6. Independence of the amplitude of the ganglionic and the preganglionic responses. Capacity-coupled amplifier. A, ganglionic record, time calibration 1 sec. B, preganglionic record, time calibration 5 msec.

stimulation from 2 to 20 per sec. (Rosenblueth, 1932). With higher frequencies (e.g., 30 to 60 per sec.) the responses decline progressively (Orías, 1932). After injections of veratrine which did not result in block the transmission at the ganglion was not significantly affected, as judged by the comparison of responses to relatively high frequencies with the normal controls, or with the responses to postganglionic stimulation at the same frequencies.

*C. Independence of the several effects of veratrine.* The action of veratrine on the spike magnitude and on the negative and positive after-potentials, described above, were independent of each other. By this is meant that any of those effects could be prominent while the others were slight, or that an increase of one of the features could correspond to a decrease of another.

The independence could be brought out by means of progressively increasing doses of the drug. Thus if small quantities (e.g., 0.1 mgm.) were successively injected, the first changes noted were an increase of the spikes and of the negative

after-potential; repetition of the responses was seen later; the characteristic sequence of changes of amplitude (decline  $\rightarrow$  increase) of the spike and the negative after-potential with repetitive stimulation appeared only with larger doses than the previous; finally, very large doses led to block of responses.

The changes of magnitude of the spike potentials with repetitive stimulation were not always parallel with corresponding changes of the negative after-potential (fig. 5). Both changes were not correlated with the amplitude of either the negative or the positive after-potentials in responses to single shocks. Finally, the after-potentials themselves varied independently, as indicated by the possibility of observing either one increase with repetitive stimulation.

D. *The preganglionic and the postganglionic axons.* In order to evaluate some of the effects registered at the ganglion a few observations were made on the action of veratrine on the preganglionic and the postganglionic axons.

In some experiments, as already mentioned, both stimulating and recording electrodes were placed on the preganglionic trunk. Veratrine was found to cause a slight increase or a decrease of the spike potentials. It markedly increased the negative after-potential. It led to the typical decrease, then increase of responses upon repetitive stimulation at relatively low frequencies. It caused repetitive discharges in response to single shocks (fig. 3A). Finally, in large concentrations it abolished all the nerve responses.

The postganglionic fibers were not studied in a similar manner, because the stretch available at the neck is only a few millimeters long. That veratrine causes repetition of these axons was seen, however, in experiments in which larger contractions than normal were recorded from the nictitating membrane in response to single shock maximal stimulation of the postganglionic axons after crushing the tip of the superior cervical ganglion. The contractions of the membrane to standard doses of adrenaline were not greater than normal, thus excluding a direct action of veratrine on the indicator.

The rate of the repetitive discharges to single shocks was approximately the same for the preganglionic and the postganglionic elements: about 300 per sec. at the beginning of the discharge. This rate is slower than that observed by Acheson and Rosenblueth (1941) in axons A: i.e., 500 to 650 per sec.

E. *Blocking effects.* Large doses of veratrine (0.5 to 2 mgm.) caused a reversible cancellation of the responses of the ganglion or the nictitating membrane to preganglionic stimulation.

The disappearance of responses was sometimes due to an abolition of the preganglionic nerve impulses, as shown by the negative records obtained from these fibers. Stimulation of the postganglionic fibers in those cases failed sometimes also to evoke membrane responses. The contractions of the membrane to injections of adrenaline were only slightly depressed. It may be inferred that veratrine can also abolish conduction in the postganglionic axons. Indeed, some of the observations showed a block of the ganglion and the postganglionic axons while the preganglionic elements were functional (fig. 7B).

In some instances (fig. 4F and G) stimulation of the preganglionic fibers failed to elicit responses in the membrane, or caused only slight contractions, while

stimulation of the postganglionic elements evoked large responses. These observations indicate that certain doses of veratrine may block only certain fibers (the preganglionic) in the tract.

The following interesting effect was frequently seen. After a given dose of

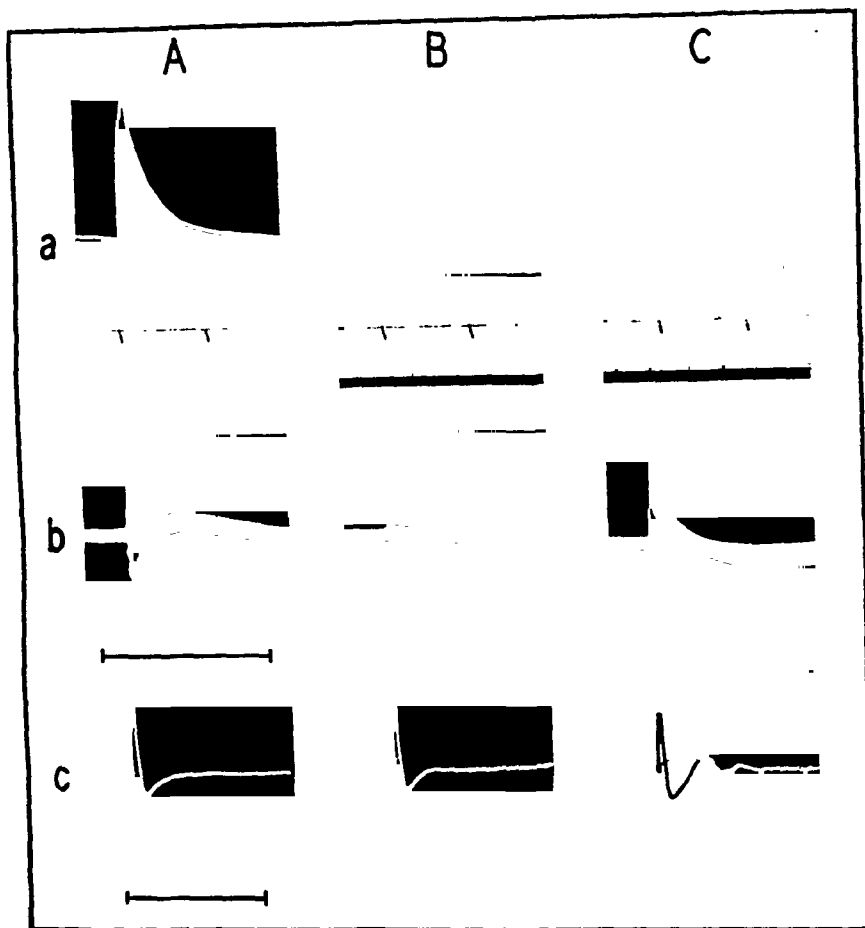


Fig. 7. Block of the postganglionic without block of the preganglionic fibers. Maximal stimulation of the preganglionic fibers. The records are as follows: *a*, mechanograms of the nictitating membrane in response to 5 sec. stimulation at the rate of 0.5 per sec.; *b*, diphasic electrograms of the superior cervical ganglion (single shock stimulation), time calibration 1 sec.; *c*, diphasic electrograms of the preganglionic fibers (single shock stimulation), time calibration 20 msec. A, before veratrine. B, 1 min. after injection of veratrine (0.5 mgm.). C, 6 min. later; strong stimulation of the postganglionic fibers failed to elicit any membrane responses. About 20 min. later the membrane began to respond and the electrograms were similar to those in C.

veratrine stimulation of the preganglionic fibers resulted in large ganglionic responses of normal latency but failed to activate the nictitating membrane (figs. 7C and 8B). Application of strong shocks to the postganglionic fibers failed likewise to cause contractions of the membrane (fig. 8D). That the smooth muscle of the membrane was responsive was shown by the satisfactory responses brought forth by intravenous injections of adrenaline (fig. 8C). It

may be concluded, therefore, that veratrine may block conduction in postganglionic axons without blocking the preganglionic fibers or the cell-bodies at the ganglion.

In no observation was there any evidence of block of transmission at the ganglion. Failure of response could always be attributed to block of the preganglionic or the postganglionic conducting elements, or both, without postulating a specific effect at the ganglionic synapses.

F. *Effects of veratrine on the nictitating membrane.* The experiments were

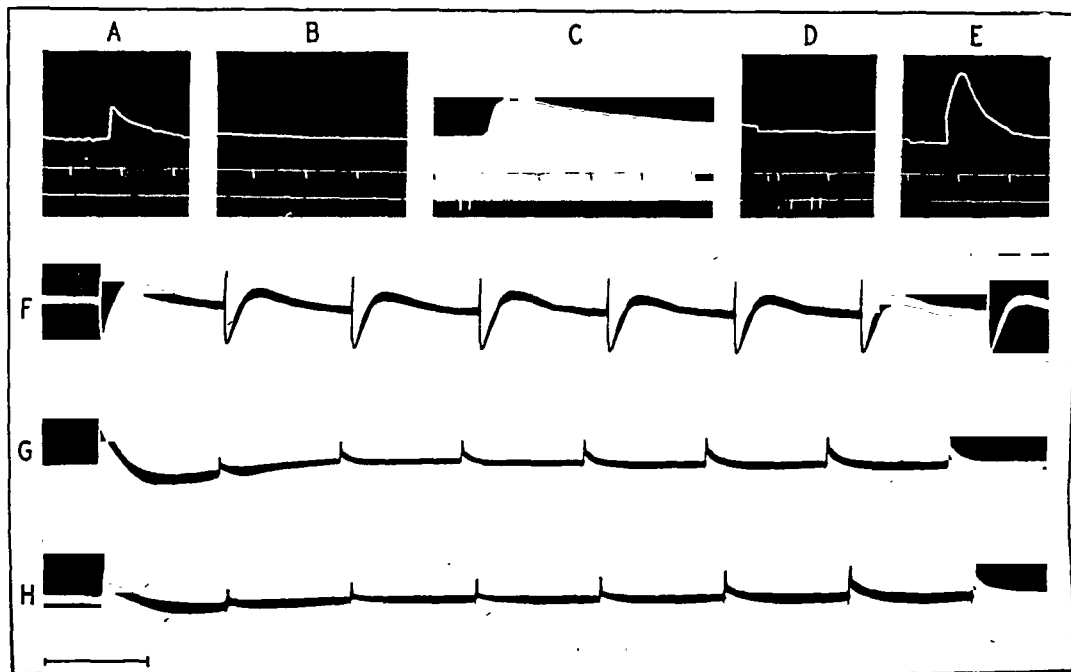


Fig. 8. Block of the postganglionic fibers without block of the ganglion. Maximal stimulation of the preganglionic fibers.

A and F, controls before veratrine. Mechanogram from the nictitating membrane and electrogram from the superior cervical ganglion (time calibration, 1 sec.). B and G, similar records 20 min. after injection of veratrine (1 mgm.).

C, response to intravenous adrenaline (20γ) 1 min. later. D, strong stimulation of the postganglionic fibers between signals 1 min. later. E and H, responses to preganglionic stimulation 10 min. later.

planned to minimize the possible action of the drug on the membrane, since the organ was meant merely to indicate ganglionic activity. In all the animals in which the membrane was used intravenous injections of adrenaline were made before and after the administration of veratrine in order to test the state of the indicator. Small or moderate doses of the drug caused slight or no changes in the responses to adrenaline. Large doses of veratrine resulted in a depression of the responses of the membrane from 20 to 50 per cent less than normal (cf. fig. 4B, E and I).

The injections of veratrine evoked a transitory contraction of the membrane

(fig. 4C). This contraction was due to a stimulating action at the ganglion, for in a few control experiments no contractions ensued upon injecting veratrine into animals after removal of the superior cervical ganglion.

DISCUSSION. I. *The effects of veratrine on the superior cervical ganglion.* Table 1 summarizes the effects of veratrine on axons, on striated muscle and on the superior cervical ganglion. The only discrepancies are in the action of the drug on the demarcation potential. The striking similarity of other effects supports the view that the conducting mechanism is identical in the three structures. Indeed, in striated muscle Rosenblueth, Wills and Hoagland (1941) came to the conclusion that the effects of veratrine could all be explained by the assumption that the drug affects exclusively the conducting system, and that it was unnecessary to postulate any significant action on the contractile mechanism.

TABLE 1

*The effects of veratrine on axons, on the superior cervical ganglion and on striated muscle*

The symbols have the following meanings: s.d., small dose, and l.d. large dose of veratrine; +, present or increased; -, decreased; ?, undetermined by the data available. The data for axons are from several sources; references will be found in Acheson and Rosenblueth (1941); those for striated muscle are from Rosenblueth, Wills and Hoagland (1941).

	AXONS	SUPERIOR CERVICAL GANGLION	STRIATED MUSCLE
Demarcation potential.....	- or +	-	+
Spike potential {s.d.....	+	++	++
{l.d.....	-	?	-
Negative after-potential.....	++	++	++
Positive after-potential.....	?	+	+
Repetition to single shocks.....	++	+	+
Alternation of responses.....	+	+	?
Decrease followed by increase of spikes and negative after-potential with repetitive stimulation.....	+	+	+
Abolition of response.....	+	+	?

The study of the action of veratrine on axons led Acheson and Rosenblueth (1941) to the conclusion that the independent variation of the demarcation and the spike potentials and of the negative after-potential denoted an independence or only a loose correlation between the processes underlying these electric manifestations. The present data on the ganglion (figs. 2, 5 and 6) support that conclusion and lead further to the inference that the positive after-potential may also vary independently of the other electric signs of ganglionic activity.

II. *The electric responses of the ganglion.* The electrograms of the superior cervical ganglion have been interpreted as manifestations of cell-body activity (Eccles, 1935a; Rosenblueth and Simeone, 1938), or else have been attributed in part to the postganglionic axons (Bishop, 1936).

The type of block by veratrine illustrated in figures 7 and 8 supports the view that the ganglion potentials are mainly of cell, not of axon origin. The records show that the postganglionic axons supplying the nictitating membrane, and



probably others supplying other structures, may be totally blocked, yet the electrograms from the ganglion are quite similar to those obtained later, when conduction is renewed in the blocked axons (cf. fig. 8G and H). It may be inferred, therefore, that some structure at the ganglion other than the postganglionic axons yields the potential changes recorded.

If that structure is the cell-body, in view of the inferences made in section I, it may be further concluded that conduction in the soma of neurons is qualitatively similar although quantitatively different from the conduction which takes place in the corresponding axon.

III. *A comparison of transmission with conduction at the ganglion.* If a synaptic transmission were a process similar to that of conduction in axons, as has been frequently suggested, then it would be expected that the action of veratrine on synapses would be similar to that which it has on axons. Indeed, since synapses are much more sensitive to the action of drugs than are axons (Sherrington, 1906), it would be expected that much smaller doses of veratrine than those necessary to affect the axons would suffice to cause marked synaptic effects.

That expectation was not fulfilled. Repetitive discharges at the ganglion were only found when the preganglionic fibers discharged repetitively (p. 702). Nor was pure synaptic block seen—i.e., absence of transmission only occurred when either the preganglionic, or the postganglionic axons, or both, had been rendered unresponsive by the drug (p. 708).

The conclusion is reached, therefore, that synaptic transmission at the ganglion is a process different from that of conduction in sympathetic axons. This conclusion is in agreement with the recent knowledge that transmission, unlike conduction, is due to liberation of acetylcholine.

#### SUMMARY

The effects of intra-arterial injections of veratrine on the superior cervical ganglion were studied in cats. The indicators used were the electric responses of the ganglion and the contractions of the nictitating membrane, upon stimulation of the preganglionic fibers.

After veratrine the electrograms of the ganglion may exhibit an increase of the spike potentials (figs. 1 and 2), of the negative after-potential (figs. 1, 2 and 7), and of the positive after-potential (fig. 2). The responses to single shocks may become repetitive (figs. 3 and 4). The successive responses to a series of stimuli at slow rate often decrease at first and increase later (figs. 5 and 6). The drug may block selectively either the preganglionic (fig. 4) or the postganglionic elements (fig. 7B). It may block conduction in the postganglionic axons without impairing the responses of the cell-bodies at the ganglion (figs. 7C and 8B, D and G).

A comparison of the effects of veratrine on axons, striated muscle, and the superior cervical ganglion (table 1) reveals a close parallelism that suggests a fundamental similarity of the conducting mechanism in the three structures. Synaptic transmission at the ganglion is not significantly affected by veratrine. It is inferred that the mechanism of transmission from neuron to neuron differs from the mechanism for conduction within a neuron.

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# THE EFFECT OF ADRENALECTOMY ON THE ABSORPTION OF HYDROGENATED COTTONSEED OIL, CORN OIL, TRIBUTYRIN AND SODIUM BUTYRATE<sup>1</sup>

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Adrenalectomy was shown early by Verzar and Laszt (1935) to result in a definite inhibition of fat absorption. Opposing these early investigators is the work of Barnes, Miller and Burr (1941) who demonstrated that adrenalectomized rats maintained in a good state of health by the administration of salt solution, absorbed corn oil and its methyl esters at the same rate as the normal controls. Recently, however, Bavetta et al. (1941) have reported that there is a decrease of approximately 24 per cent in the rate of absorption of hydrogenated cottonseed oil by salt-treated adrenalectomized female rats as compared with normals. It was further shown that the absorption of this fat could be restored to normal by the administration of cortin. Since the appearance of this paper, Barnes, Rusoff and Burr (1942) have reported additional experiments where it was found that emulsified hydrogenated cottonseed oil was the only fat the absorption of which was depressed following adrenalectomy. These investigators noted no significant decrease in absorption of corn, olive or hydrogenated cottonseed oil or of mutton tallow resulting from ablation of the adrenals. It appears unreasonable that this inhibition in fat absorption would be limited only to a special type of fat. It seemed desirable therefore to investigate the problem still further with the hope of ascertaining, if possible, the reason for these divergent results.

In the present study we have compared the absorption of hydrogenated cottonseed oil, corn oil, tributyrin and sodium butyrate in normal and adrenalectomized animals.

**METHODS.** The tests on corn and hydrogenated cottonseed oils were carried out over an 8 hour period as employed by Barnes et al. (1942) instead of the 3 hour interval which we had previously used. However, since similar results were obtained in the 3 and 8 hour tests, the experiments with tributyrin were carried out only over a three hour period while those with sodium butyrate were terminated at the end of 90 minutes. The procedure employed for the study of the absorption of the neutral fats was similar to that in the earlier report (1941) while the method used in the tests on sodium butyrate was the same as that described by Deuel et al. (1941). After subtraction of the value of the ether-

<sup>1</sup> These data are from a thesis to be presented by Lucien Bavetta to the Graduate School of the University of Southern California in partial fulfillment for the degree of Doctor of Philosophy. The results were presented before the meeting of the Southern California Section of the Society for Experimental Biology and Medicine, March 17, 1942.

soluble material found in the intestines of control rats fasted a similar period, the weights of the fats removed from the intestine are corrected for a recovery of 93.6 per cent for the hydrogenated cottonseed oil, 94.4 per cent for corn oil, 90.2 per cent for tributyrin and 92.5 per cent for sodium butyrate. These values represent the average percentage of each fat recovered in 10 experiments when known amounts of the different fats were given and the gastro-intestinal tracts immediately removed.

RESULTS. The results are summarized in table 1. It was found that the

TABLE 1

Summary table of absorption of hydrogenated cottonseed oil, corn oil, tributyrin, and sodium butyrate by normal (N) and adrenalectomized (A) female rats

OIL FED	SERIES OF TESTS	ABSORPTION PERIOD	NUM- BER OF TESTS		BODY WEIGHT IN GRAMS		ABSORPTION IN MCM. PER 100 SQ. CM. PER HOUR			TITRATION IN CC. 0.1 N NaOH*		
			N	A	N	A	N	A	M.D.: S.E.M.D.†	N	A	M.D.: S.E.M.D.†
Hydrogenated cottonseed	Bavetta et al. (1941)	3 hrs.	17	31	117	146	36.3 ± 1.0	27.6 ± 1.6	4.53 (90%)	3.0 ± 0.3	7.9 ± 0.2	13.62 (100%)
Hydrogenated cottonseed	Deuel, Hall- man, Leon- ard (1940)	6	10			152	39.7 ± 1.8					
Hydrogenated cottonseed	Present tests	8	12	10	86	110	42.8 ± 1.4	28.7 ± 3.6	3.65 (90%)	2.6 ± 0.1	5.7 ± 1.7	1.84 (100%)
Corn oil	Present tests	8	9	11	93	113	44.9 ± 1.6	29.3 ± 3.7	3.87 (91%)	2.1 ± 0.6	5.2 ± 0.9	2.88 (89%)
Tributyrin	Deuel, Hall- man (1940) ‡	3	10			190	65.0 ± 2.5					
Tributyrin	Present tests	3	11	16	108	117	69.1 ± 3.7	65.8 ± 1.8		0.6 ± 0.3	0.5 ± 0.1	
Sodium butyrate	Deuel, Hall- man, Reif- man (1941)	1	18			155	39.7 ± 1.5					
Sodium butyrate	Present tests	1.5	16	14	122	103	45.0 ± 2.6	42.8 ± 2.3				

\* Including Standard Error of Mean.

† Mean Difference: Standard Error of Mean Difference. The figures in parentheses are the percentage of tests of series having lower average which do not overlap higher average. A value of 90 per cent is considered significant.

‡ Male rats were used.

rate of absorption of hydrogenated cottonseed oil based on the present 8 hour tests is practically identical with the earlier results where the 3 hour interval was employed. Furthermore, it is to be noted that the absorption of corn oil after adrenalectomy was inhibited to the same degree as that of hydrogenated cottonseed oil. In addition the increase in the fatty acid present in the gut contents of rats fed corn oil was as great as those receiving hydrogenated cottonseed oil. On the other hand no decrease in absorption occurred after removal of the adrenals in the case of tributyrin or with sodium butyrate.

DISCUSSION. The present experiments further support the earlier work of Verzar and Laszt (1935) in showing that the adrenal glands are involved in the

normal absorption of fat. The decrease in absorption of hydrogenated cottonseed oil after adrenalectomy was somewhat greater than noted in the previous report and amounted to 33 per cent. A similar inhibition in the absorption of corn oil was found (35 per cent) after removal of the adrenals.

The depression in absorption rate after adrenalectomy both with the hydrogenated cottonseed and corn oils is statistically significant based either on the comparison of mean difference to standard error of mean difference or by the degree of overlapping. Moreover, further support of the alteration in fat absorption which results after the removal of the adrenals is to be found in the fact that a marked and statistically significant increase in the fatty acid content in the gut obtains after both fats. There is no evidence that different factors control the absorption of corn oil and hydrogenated cottonseed fat as indicated by Barnes et al. (1942).

Although in our earlier experiments, the length of the absorption period was shorter and the dose of fat smaller than employed by Barnes et al. (1942), in the present tests these procedures were identical. Somewhat different technics were used for recovery of the unabsorbed fat from the gastrointestinal tract by Barnes et al. (1942) than employed by us. However, this can not explain why the results of these investigators on normal animals are in agreement with ours while only the values on the adrenalectomized animals are not in harmony. In the normal rats the rate of absorption per 100 sq. cm. per hour obtained by Barnes et al. (1942) and by us was 39.8 mgm. and 42.8 mgm. respectively for hydrogenated cottonseed oil; for corn oil the values were 42.4 mgm. and 44.9 mgm. respectively. The most probable explanation for the discrepancies is that Barnes et al. (1942) employed much larger and older rats. Their animals weighed somewhat over 300 grams while the average weight of our rats in the present tests approximate 110 grams. It is well known that the cortical deficiency is much more critical in younger animals.

On the other hand the absorption of tributyrin and of sodium butyrate were not influenced by removal of the adrenals. One must conclude from such data that the absorption of such water-soluble acids as butyric probably does not require adrenal activity.

#### SUMMARY

A definite inhibition in the absorption of both hydrogenated cottonseed and corn oils was noted in adrenalectomized rats. Furthermore, larger amounts of fatty acids accumulated in the intestines of such animals as contrasted with the normal. This would indicate that the adrenal glands play a rôle in the absorption of the longer chain fatty acids. However, the absorption of tributyrin and of sodium butyrate was unaffected by adrenalectomy which suggests that the absorption of the water soluble fatty acids is probably not dependent on adrenal activity.

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*Addendum.* In more recent experiments it has been found that the absorption of sodium butyrate is significantly lowered by adrenalectomy when the operated animals are maintained on water alone rather than on salt solution (as administered to all other adrenalectomized rats used in the present tests). The average value in 11 experiments was 33.4 mg. per 100 sq. cm. per hr. which is significantly lower than the value found in normal animals (M.D.:S.E.M.D. is 3.21). However, this effect is a secondary one related to altered salt balance with attendant hemoconcentration and concomitant circulatory disturbances rather than to a direct effect of the adrenals on the absorption of butyric acid.

# THE PARATHYROIDS AND THE CLEARANCE OF INORGANIC PHOSPHATE<sup>1</sup>

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A number of investigators have suggested that the primary effect of the parathyroid hormone is exerted upon the kidney and affects the ability of the kidney to excrete phosphate (1, 2, 3). If this be true the parathyroidectomized dog or the animal treated with parathyroid hormone should show changes in the phosphate clearance. The experiments of Logan (4) show an immediate increase in the amount of inorganic phosphate excreted per hour in the urine after administration of the hormone although the figures which he gives are not sufficient to calculate the clearance.

Pitts (5) reported that the phosphate clearance is a function of the plasma concentration, approaching the xylose clearance only at high plasma concentrations, about 9 mM. These xylose figures have been converted to creatinine values by Smith (6) and he shows that at levels higher than 9 mM the values for phosphate approach but never exceed the creatinine clearance. Pitts made the assumption that the serum phosphate was in major part if not entirely filterable at the glomerulus. Harrison and Harrison (7) investigated this point and reported that 76 to 100 per cent of the inorganic phosphate was in filterable form after injection of phosphate, reaching 100 per cent about an hour after injection.

In the experiments reported here we have made a comparison of the clearance of phosphate and of creatinine in normal dogs, in dogs after parathyroid hormone injections and in parathyroidectomized animals.

**METHODS.** Female dogs were trained for clearance determinations. The animals were maintained on a diet of constant composition with casein as the protein and with adequate amounts of vitamins and minerals (8). A mixture of mono- and disodium phosphates at a pH within the range of blood and usually of molar concentration was injected into the small saphenous vein at a rate sufficient to maintain high levels of phosphate in the blood during the experiment. After the desired level was reached it was possible to keep the level fairly constant for several clearance periods. Some of the clearances were done after a single large injection and on a falling blood concentration. Blood samples were removed from the jugular vein at the midpoint of the period or at more frequent intervals if the blood concentration were changing. Creatinine was given either by mouth before the experiment began or was injected in solution with the phosphate.

The injection of 100 or 200 units of Parathyroid Extract, Lilly, was made

<sup>1</sup> This work was partially supported by a grant to one of us (M. F.) from the Committee on Scientific Research of the American Medical Association.

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subcutaneously. To test the effect of the hormone, analyses were made on hourly urine specimens and an increase in phosphate was always obtained within the second hour. Clearances were carried out 3 hours or more after the injections. In a few experiments the interval was prolonged to 16 or 24 hours after hormone administration. Clearances were determined on the operated animals in the same way. These experiments were difficult to perform and it was impossible to maintain the standard dietary regime with the parathyroidectomized

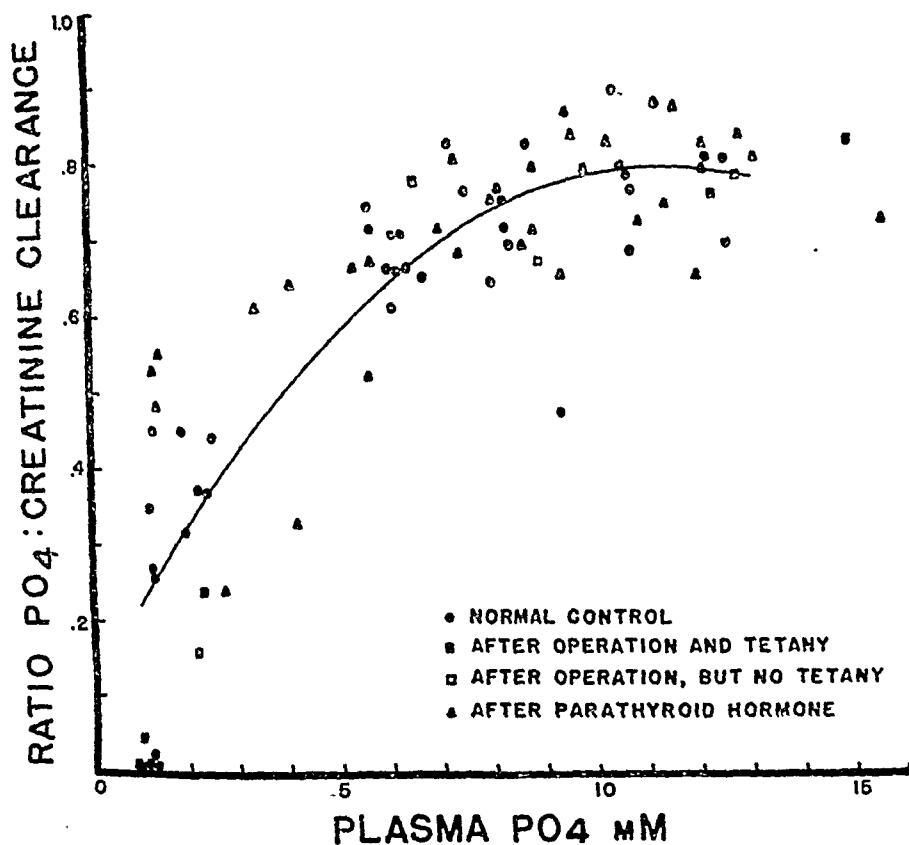


Fig. 1. The non-linear correlation for the normal points was determined by the method of least squares.<sup>3</sup> All of the experimental points lay within 3 standard errors and all but 4 points within 2 standard errors of the normal curve. A new calculation including all the determinations was made to have the advantage of a larger number of figures in determining the line. The equation for the curve is:

$$\text{PO}_4: \text{Creatinine clearance} = 10.580 (\text{Plasma PO}_4 \text{ in mM}) - 0.546 (\text{Plasma PO}_4 \text{ mM})^2.$$

Standard error = 11.05; index of determination = 0.77; index of correlation = 0.88.

animals. The number of these experiments successfully carried out is not large but we include them since in spite of the experimental difficulties the agreement with the other experiments is good.

Creatinine was determined by the Folin-Wu method as modified for the Evelyn photoelectric colorimeter. The Fiske and Subbarow method for inorganic phosphorus was used in the photoelectric modification.

RESULTS. The chart shows the relationship between the phosphate: cre-

<sup>3</sup> Our thanks are due to Dr. Versa V. Cole who made the statistical analysis for us.



atinine clearance ratio and the plasma phosphate concentration in more than 80 determinations, 39 on normal dogs, 33 on dogs after hormone administration and 9 after parathyroidectomy. Two of the latter are on an animal which did not show any signs of tetany after operation, which is indicated in figure 1. In calculating the clearance figures no corrections have been made for non-filterable phosphate as we assume that any error thus introduced will influence control

TABLE 1

*Dog Q, 14.4 kgm. January 13, 1942; 69.6 cc. M phosphate, pH 7.42, containing 3.6 grams creatinine, injected during 2 hours*

TIME	URINE	PLASMA PO <sub>4</sub>	CLEARANCE		RATIO P/C
			Creatinine	Phosphate	
min.	cc./min.	mM	cc.	cc	
25.6	0.98	6.0	49.7	30.2	0.61
23.0	1.70	8.11	53.4	40.2	0.75
26.3	2.02	10.5	49.4	38.5	0.78
20.0	2.50	10.45	54.5	43.1	0.79

February 3; 76.7 cc. M phosphate, pH 7.3, containing 3.8 grams creatinine, injected during 1 hour and 50 minutes, 17 hours after 200 units parathyroid extract

30.0	6.59	6.94	58.1	41.2	0.71
20.0	3.34	8.74	56.6	45.0	0.79
21.1	2.49	9.72	56.0	44.2	0.79
21.0	3.04	10.20	57.9	47.6	0.82

February 17; 71.8 cc. M phosphate, pH 7.4, containing 3.6 grams creatinine, injected during 1 hour, 4 hours and 24 minutes after 200 units parathyroid extract

20.0	3.58	13.05	56.7	45.4	0.80
20.0	2.96	12.02	57.8	47.5	0.82

February 26; thyroids and parathyroids removed; February 28, tetany, treated with calcium lactate; March 2, 83.4 cc. M phosphate, pH 7.42, containing 4.17 grams creatinine, injected during 1 hour and 18 minutes. Symptoms of extreme tetany developed during the injection. Control Ca, 5.4 mgm.; at end of injection, 3.8 mgm. per 100 cc.

20.0	2.37	12.21	42.5	32.2	0.76
39.4	2.65	14.85	44.0	36.1	0.82

and experimental periods alike. No significant differences can be noted between the groups.

Table 1 gives representative protocols of four experiments on one dog. This was the only animal which was used for all three types of experiments, the others being used after hormone injections or after parathyroidectomy.

DISCUSSION. Under our experimental conditions there is no apparent alteration in the clearance of phosphate by the kidney after removal of the parathyroids or after administration of parathyroid hormone.

The results after injection of the hormone might have two explanations: First, that the hormone effect is not exerted primarily upon the ability of the kidney to excrete phosphate; or second, that injection of phosphate inhibits hormone action upon the kidney. Neufeld and Collip (9) have shown that after treatment with parathyroid extract normal dogs show no rise in serum calcium during phosphate injections but that after the injection was stopped the calcium did increase. Serum calcium determinations in our experiments confirmed this result. This raises the question as to whether or not the effect of the hormone can be exerted upon the kidney if the serum calcium cannot increase, a question which is involved with the possibility of independent action of calcium and phosphate ions. Tweedy (3) suggests the possibility that they can act independently in his report of a rise in plasma inorganic phosphate in dogs with complete renal insufficiency whether or not a hypercalcemia had been produced by parathyroid extract. If this be true the presence or absence of hypercalcemia in our experiments should not interfere with any influence of the hormone upon the phosphate excretion by the kidney.

The parathyroidectomized dogs gave results no different from the other groups. In these animals the injected phosphate should accentuate the lack of hormone by lowering the calcium and raising the phosphate in the blood. No effect upon the clearance ratios was observed.

#### SUMMARY

Phosphate and creatinine clearances were determined during or after the intravenous injection of phosphate in normal dogs, in parathyroidectomized dogs and in dogs after administration of parathyroid extract. Under the conditions of our experiments a lack or an excess of parathyroid hormone produced no demonstrable effect upon the capacity of the kidney to excrete phosphate.

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# THE SALIVATORY MOTOR NUCLEI IN THE MONKEY<sup>1</sup>

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The somewhat similar position of the Edinger-Westphal nucleus and the dorsal motor nucleus of the vagus, in the central gray matter ventral to the cerebral aqueduct and fourth ventricle, suggests the presence in the brain stem of a paramedian, general visceral motor column, in which the nuclei giving rise to the preganglionic outflows to the salivary glands might logically be expected to be situated (Kimmel, 1940). Experimental evidence which can be interpreted as favoring this localization is that of Miller (1913), who elicited salivary flow from paramedian stimulation of the floor of the fourth ventricle in the decerebrate cat.

The studies of Kohnstamm (1902, 1903), Kohnstamm and Wolfstein (1907), Yagita and Hayama (1909), and Yagita (1909), based upon the examination of central chromatolysis after peripheral nerve section, suggested, however, that the salivatory nuclei might be located in the lateral part of the medulla, more in relation to the special visceral efferent column (motor nuclei of the fifth and seventh nerves and nucleus ambiguus) innervating striated musculature derived from the branchial arches. The results of these authors have been widely accepted, but an examination of their papers reveals such disagreement regarding the type and location of the chromatolytic cells under consideration, and such difference of opinion concerning the crossed or ipsilateral distribution of their efferent fibers, as to suggest that further investigation is desirable.

The positive results obtained by Miller (1913) from activating the floor of the fourth ventricle suggested that an examination of the location of points in the interior of the medulla yielding salivary responses to discrete electrical stimulation might provide information which would be of aid in identifying the salivatory nuclei. The excitability of appropriate bulbar levels in a series of 8 monkeys was, therefore, explored with the Horsley-Clarke technique (Ranson, 1934). The results are in general agreement with those of similar studies in the cat by Chatfield (1941) and Wang (1942), which appeared after the experimental aspect of the present investigation had been completed.

**METHOD.** In each of 8 monkeys under chloroform anesthesia (65 mgm. per kilo) the interior of the medulla was electrically stimulated in a systematic manner with small bipolar electrodes oriented with the horizontal electrode carrier of the Horsley-Clarke instrument. In 5 animals, stimulation was carried out in planes slightly oblique from the transverse, and in 3 cases in sagittal planes. The location of the points stimulated was determined by microscopic examination of Weil stained serial sections through the region of the brain explored.

<sup>1</sup> Aided by a grant from the Rockefeller Foundation.

<sup>2</sup> Medical Fellow of the National Research Council.

Stimulation was applied for 30 second periods and consisted of thyatron regulated condenser discharges at a frequency of 30 per second and at intensities between 1 and 30 volts, voltages of 2.5 (2 animals), 5.5 (4 animals) and 10 (2 animals) being employed throughout responsive regions. Exploration was confined to one-half of the medulla, and the parotid ducts of both sides were cannulated in the cheek and the submaxillary ducts of both sides in the mouth, a short distance from their termination. The cannulae consisted of lengths of 20 gauge, blunt tipped, syringe needles. The drops of saliva were observed visually and the time of their fall recorded on moving kymograph paper with signals.

**RESULTS. Salivary responses.** The experiments revealed a responsive segment of the medulla the stimulation of which readily caused saliva flow from the ipsilateral submaxillary and parotid glands. Responses of varying magnitude were obtained and in the case of the submaxillary reactions as many as 10 drops resulted from the 30 second period of stimulation. Secretion from the larger parotid gland was commonly greater and 30 drops represented the maximal flow. In favorable locations, sizable responses could be obtained with stimulus intensities of only 1 volt, suggesting, by analogy with data from peripheral nerves, that if the excitation of fibers was responsible, these were not of the small, unmyelinated variety.

The temporal course of the flow from the two glands was similar. Drops first fell from the cannula 3 to 6 seconds after commencement of stimulation, the latency of parotid flow tending to be shorter than that of the submaxillary. A steady rate of flow might be maintained during the period of stimulation, or in the case of some parotid responses, the flow might be more rapid during the first half of stimulation. Commonly, in large responses, 2 or 3 drops fell at increasing intervals during a period of 5 to 20 seconds after the conclusion of stimulation.<sup>3</sup> The profuse flow and fluid appearance of the drops suggested their elaboration as a result of parasympathetic stimulation, and the results from 1 animal in which both cervical sympathetic trunks were sectioned at the start of the experiment did not differ from the others.

**Distribution of reactive points.** The excitable bulbar region from which salivary responses were obtained comprised the dorsal midline area between the genu of the facial nerve and the rostral end of the hypoglossal nucleus, and extended laterally and ventrally through the reticular formation to the lateral margin of the medulla at the levels of exit of the facial and glossopharyngeal nerves. Though overlap existed, it was evident that submaxillary responses were obtained predominantly from the rostral portion of the excitable region, while parotid responses were excited predominantly from the caudal part. A general picture of the extent of the responsive field, as seen in transverse section, has been obtained by combining the results from 3 experiments, in which exploration was conducted in the planes shown in figure 1. A supplemental, sagittal

<sup>3</sup> Following punctures into excitable regions, occasional single drops of saliva sometimes fell at intervals for long periods. To avoid confusing these with minimal responses to stimulation at subsequent points, all single drop effects have been disregarded in this presentation.

view has been provided by combining the results from 3 other experiments, in which stimulation was carried out in the planes shown in figure 2.

It can be seen that responses of the ipsilateral submaxillary gland, denoted by circles, were elicited from the dorsomedial portion of the medulla at the level of

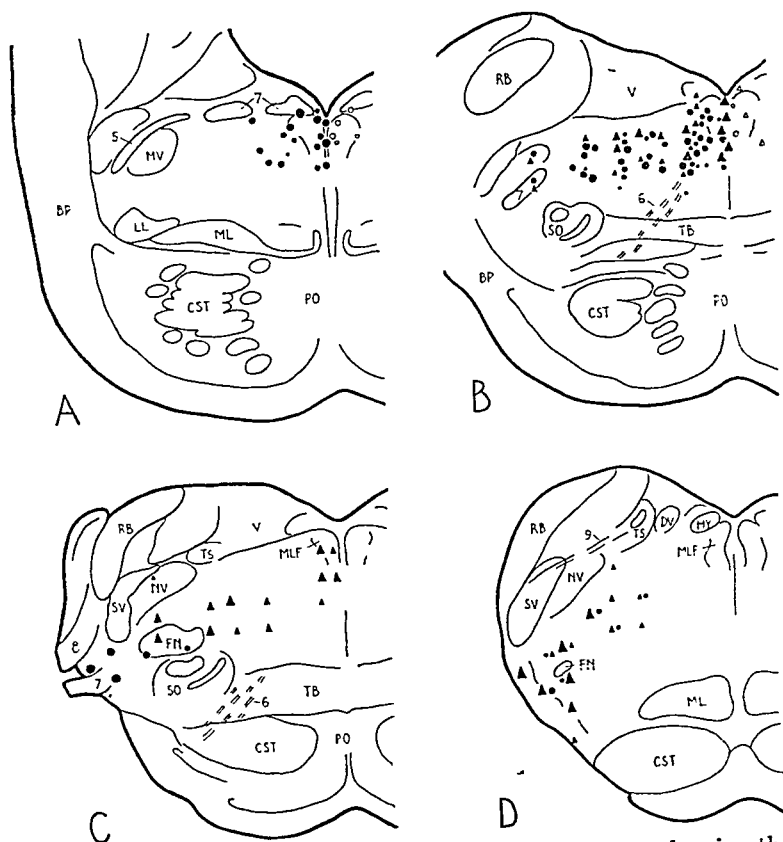


Fig. 1. Sections through the left half of the brain of the monkey, showing the distribution of points yielding salivary flow on stimulation. Responses of the ipsilateral submaxillary gland are indicated by solid circles, and those of the ipsilateral parotid gland by solid triangles. Contralateral effects are denoted by open symbols on the right. The size of the symbol is roughly proportional to the volume of flow. The planes are at intervals of about 2 mm. and deviate from the transverse to the extent that the ventral surface is 2 mm. farther rostral than the dorsal. Abbreviations are as follows: *AN*, abducens nucleus; *BP*, brachium pontis; *CST*, corticospinal tract; *DV*, dorsal motor nucleus of vagus; *FN*, facial nucleus; *HY*, hypoglossal nucleus; *IO*, inferior olive; *LL*, lateral lemniscus; *ML*, medial lemniscus; *MLF*, medial longitudinal fasciculus; *MV*, motor fifth nucleus; *NV*, nucleus of spinal fifth tract; *PO*, pons; *RB*, restiform body; *SO*, superior olive; *SV*, spinal nucleus of spinal fifth tract; *TB*, trapezoid body; *TS*, tractus solitarius and nucleus; *V*, medial vestibular nucleus; *5*, motor fifth nerve; *6*, abducens nerve; *7*, facial nerve; *8*, eighth nerve; *9*, glosso-pharyngeal nerve.

and behind the genu of the facial nerve (fig. 1 A and B; fig. 2 A), and responsive points were distributed from the raphe laterally and ventrally through the reticular formation (figs. 1 and 2 B) to the region of exit of the seventh nerve (figs. 1 and 2 C).

Responses of the ipsilateral parotid gland, denoted by triangles, were obtained from the dorsomedial portion of the medulla behind the facial genu and ahead of the hypoglossal nucleus (fig. 1 B and C; fig. 2 A), and responsive points were distributed from the trapezoid laterally and ventrally through the reticular formation (fig. 1 B and C; fig. 2 B) to the lateral aspect of the medulla at the level of the ninth nerve (fig. 1 D; fig. 2 C). Here the responsive points are seen to be separated from the afferent glossopharyngeal fibers passing to the tractus solitarius by the extent of the spinal fifth tract and its nucleus, and the distribution of responses suggests the presence of a motor root of the ninth nerve located some distance ventral to the sensory root (fig. 1 D; fig. 2 C).<sup>4</sup>

A few responses from the contralateral salivary glands were elicited from dorsomedial positions at the levels of figure 1 A and B (open symbols on right side).

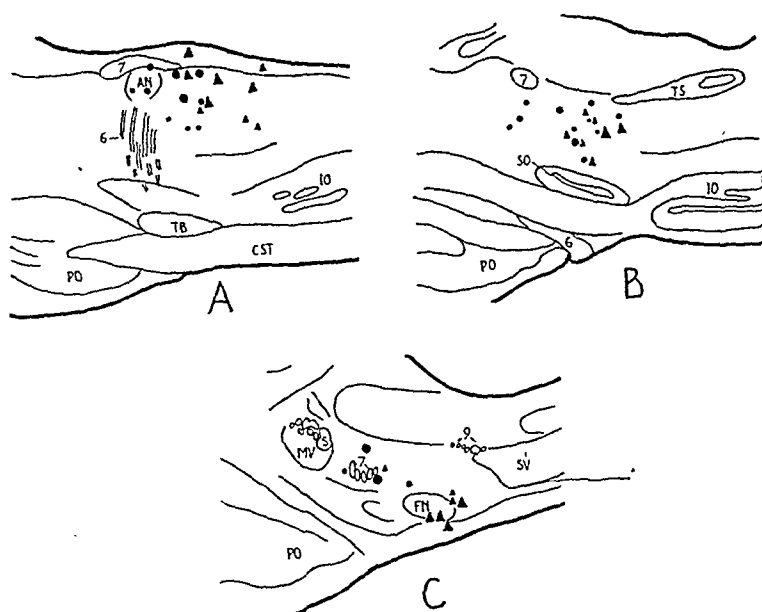


Fig. 2. Sagittal sections through the brain of the monkey, showing the distribution of points yielding salivary flow on stimulation. Symbols as in figure 1.

They may indicate the presence of a small number of crossed fibers, or they may be due to current spread to the opposite side of the medulla.

Ipsilateral lacrimation was observed in 1 experiment from dorsomedial points yielding submaxillary gland responses. Mucous secretion from the ipsilateral half of the mouth was noticed in 2 instances, but because of gradual accumulation, the precise neural region responsible for its elaboration could not be determined.

It does not seem possible to attribute the salivary responses elicited in these experiments to the activation of long pathways ascending or descending through the brain stem, for stimuli in planes 2 mm. anterior or posterior to the excitable

<sup>4</sup> What appears to be an efferent glossopharyngeal rootlet can be identified on inspection of the ventrolateral aspect of the monkey's medulla, and is situated 1 mm. or more ventrad of the main ninth root (see also Tarlov, 1940).

region with intensities as high as 30 volts failed to induce salivary flow. Neither does it seem possible to ascribe the reactions to reflex salivary flow, resulting from excitation of sensory roots or secondary afferent systems. The repeated stimulation of such bulbar afferents as the spinal fifth tract and its nucleus, or the tractus solitarius and its nucleus, never yielded salivary responses of more than 1 or, rarely, 2 drops in 30 seconds. It would appear that salivary reflexes were either suppressed by the anesthesia (see Miller, 1913) or that the intensity of the shocks employed was too low or of too brief duration, to activate their afferent fibers.

*Location of the salivatory nuclei.* If the responses elicited in these experiments are to be attributed to the stimulation of the salivatory motor nuclei and their efferent root fibers, as seems most likely to be the case, it is pertinent to enquire where in the excitable field the motor nuclei are located and where the efferent fibers. The distribution of responsive points in the dorsomedial portion of the medulla can be interpreted as indicating that the salivatory nuclei, like the Edinger-Westphal nucleus and the dorsal motor nucleus of the vagus, are situated in or near the paramedian gray matter beneath the central ventricular canal. If this be the case, the more lateral responses can be attributed to the activation of efferent salivatory rootlets passing to their exits in the seventh and ninth nerves. It seems fully as possible from the present results, however, though not from analogy with other cranial preganglionic systems, that the salivatory motor nuclei might be located more laterally and ventrally in the medulla (Kohnstamm and Yagita, see above). If this were the case, the presence of the dorsomedial responses makes it necessary to postulate that the efferent salivatory fibers possess a genu in their passage from the medulla, analogous to that of the facial nerve. The results of the present experiments do not permit one to decide which of these possibilities is correct.<sup>5</sup>

**DISCUSSION.** The observation by Corbin, Harrison and Wigginton (1941) of flushing, turgidity and pseudomotor contracture in the tongue, with accompanying salivation and sometimes lacrimation, resulting from stimulation of the region of the intramedullary course of the seventh nerve in the cat, suggests that vasodilator and possibly other parasympathetic innervation proceeds from central neural regions at least closely adjacent to those activating salivary flow. The vasodilatation of the pial vessels which may be induced by stimulating the facial nerve near the medulla (Forbes and Cobb, 1938) indicates that a parasympathetic innervation of the cerebral vessels proceeds from the same bulbar segment, and in all likelihood arises from a part of the same collection of central preganglionic neurons, innervating the lacrimal and salivary glands and the vessels of the oral mucosa.

This upper bulbar collection of preganglionic neurons would appear, therefore,

<sup>5</sup> Gutierrez-Noriega (1942) has encountered salivary flow after injection of metrazol or strychnine into the vicinity of the dorsal part of the facial nucleus of unanesthetized, decerebrate cats. The flow may, however, be a reflex one resulting from the excitant action of the drugs on the adjacent cells of the nucleus of the spinal fifth tract, the injection of which at more caudal levels also results in salivation.

to occupy an intermediate position, both in functional importance and in topographic relation, between the Edinger-Westphal nucleus, whose influence is confined to the interior of the eyeball, and the dorsal motor nucleus of the vagus from which originates the parasympathetic innervation of the thoracic and abdominal viscera. The designation of this upper bulbar collection, which, as far as evidence is available, appears to supply the entire parasympathetic innervation of the head exclusive of the eyeball, as the "salivatory" nucleus would seem an understatement of its functional rôle.

#### SUMMARY

A study in the monkey of the distribution of medullary points the stimulation of which yields salivary flow has revealed an excitable region comprising the dorsal midline area between the genu of the facial nerve and the hypoglossal nucleus, and extending laterally and ventrally through the reticular formation to the exits of the seventh and ninth nerves. While overlap exists, responses of the submaxillary gland are elicited predominantly from the rostral part of the excitable area and those of the parotid gland from the caudal part. The reactions are almost entirely ipsilateral, and are ascribed to the activation of the salivatory motor nuclei and their efferent root fibers.

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# THE NATURE OF THE S COMPLEX OF THE ELECTROCARDIOGRAM<sup>1</sup>

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The S complex of the electrocardiogram is formed by an extension of the descending limb of R and by a subsequent upward deflection to the base line. No adequate explanation for the factors entering into the formation of this complex is at present available.

Previous studies from this Laboratory have afforded evidence for the conclusion that lead I records summation of electrical events from the surfaces of the anterior left and posterior right ventricles; while lead III similarly records from the anterior right and posterior left ventricles (1). It would be expected therefore that  $S_1$  should arise from the interaction between the anterior levocardiogram and the posterior dextrocardiogram. The initial portion of  $S_1$  is a downstroke and would be expected to be derived from the electrical activity of a portion of the anterior left ventricular surface; the upstroke would be expected to be formed by late electrical activity of a portion of the right posterior ventricle. Likewise,  $S_3$  would be formed by interaction of initial electrical activity of a portion of the surface of the posterior left ventricle and late activity of a portion of the surface of the anterior right ventricle. In this paper, we report experimental verification of these inferences as to origin of  $S_3$ . The rarity of  $S_1$  in the dog as prepared for these experiments has prevented a satisfactory study of the nature of  $S_1$ .

**METHODS.** Thirteen dogs, prepared as previously described (2), were used. Three methods were employed, which also have been previously described; surface applications of KCl (2), thermal applications (3), and the study of S in the ventricular extrasystole (4).

**RESULTS AND DISCUSSION.** *Influence on  $S_3$  of KCl applications.* The application of KCl to the posterior surface of the left ventricle results in the disappearance or diminution of  $S_3$  (fig. 1A 2). When KCl is applied to the anterior surface of the right ventricle, the downstroke of  $S_3$  is greatly increased in amplitude, becoming the downstroke of a levocardiogram while the immediate upstroke which returns  $S_3$  to the base line diminishes or disappears.

Application of KCl to the anterior surface of the right ventricle may be considered to have abolished or reduced the contribution of this region to the electrocardiogram of lead III. The disappearance of the upstroke of  $S_3$  and the augmentation of its downstroke indicates that the dextrocardiogram from the anterior right ventricle was responsible for the termination of the downstroke of  $S_3$  and for its return to the isoelectric line.

The disappearance of the downstroke of  $S_3$  when the posterior levocardiogram

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<sup>2</sup> Fellow of the Dazian Foundation.

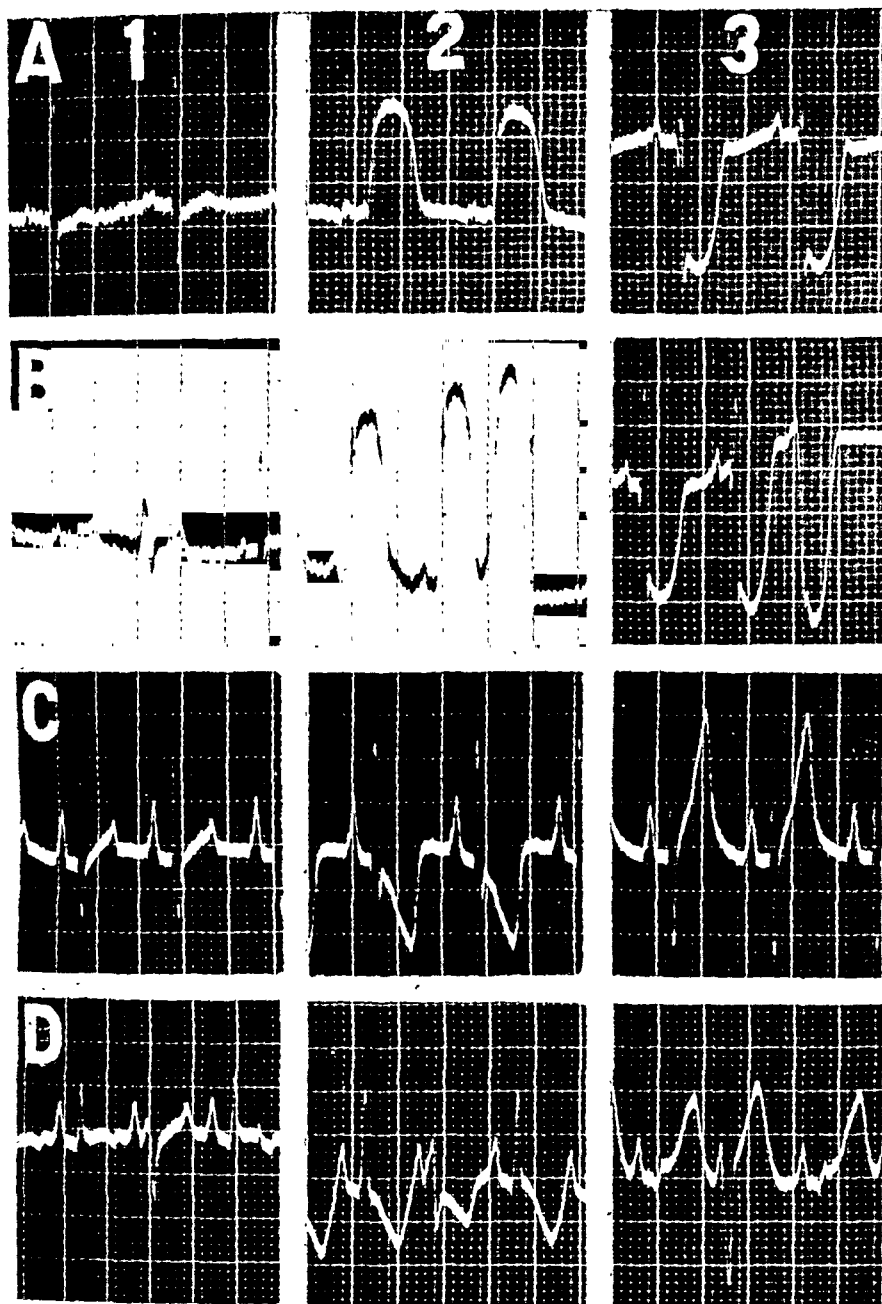


Fig. 1. Modification of S in lead III. A. Dog. 10/15/41, 169 kgm. 1. Control, lead III. 2. M/5 KCl on posterior surface of left ventricle. Abolition of S. 3. KCl on anterior surface of right ventricle. Downstroke of S is extended, with disappearance of its upstroke. B. Dog. 10/6/41. 1. Control, lead III with extrasystole elicited from point 1.0 cm. to left of the anterior septum midway between base and apex. Extrasystole as well as normal complex shows S. 2. M/5 KCl on posterior surface of left ventricle. Abolition of S in extrasystole as well as normal complex. 3. KCl anterior surface of right ventricle. Extension of down stroke and disappearance of upstroke of S in extrasystole and normal complex. C. Dog. 5/23/41. 8.1 kgm. 1. Control, lead III. 2. Cooling ( $0^{\circ}\text{C}$ ) left posterior and warming ( $48^{\circ}\text{C}$ ) right anterior surfaces of ventricles. Increase in R and decrease in S. 3. Warming posterior left and cooling anterior right ventricles. Decrease in R and considerable increase in S. Consistent T wave changes. D. Dog, 10/8/41. 13.4 kgm. 1. Control, lead III. Extrasystole elicited from point 1.0 cm. to left of anterior septum, showing prominent S and a small R. 2. Heating right anterior or cooling the left posterior ventricles. R increased and S decreased. 3. Warming the left posterior and cooling the right anterior ventricles. S greatly increased. Consistent T wave changes. Consistent alterations in R in the normal complexes.

was abolished or diminished by potassium application, supports the view that the downstroke of  $S_3$  is contributed by the posterior levocardigram.

*The influence on  $S_3$  of heating and cooling the ventricles.* Further proof that  $S_3$  is formed by the interaction of a posterior levocardigram and an anterior dextrocardiogram, is derived from heating and cooling these surfaces. Heating the anterior surface of the right ventricle and cooling the posterior surface of the left ventricle, should make that part of the right ventricle responsible for the upstroke of  $S_3$  discharge earlier with respect to the left ventricle and decrease the amplitude of  $S_3$ . Conversely cooling the anterior right ventricle and heating the posterior left ventricle should make that part of the right ventricle responsible for the upstroke of  $S_3$  discharge later with respect to the posterior left ventricle and increase the amplitude of  $S_3$ . Experimental results fully confirmed these inferences; heating the anterior right ventricle and cooling the posterior left ventricle reduced the amplitude of  $S_3$ , while cooling the anterior right ventricle and heating the posterior left ventricle increased  $S_3$  (fig. 1C, 2 and 3).

*$S_3$  in the extrasystole.* The anterior septal extrasystole shows an initial downstroke in lead I and an initial upstroke in lead III. The initial portion of extrasystoles elicited from the center of the left ventricle is downward in both leads I and III. Extrasystoles from points intermediate between the above regions show transitional stages in lead III. In extrasystoles originating just to the left of the anterior septum the main initial deflection remains upright, but a small wave corresponding to S appears. As the point of stimulation is moved further to the left toward the center of the left ventricle, the S-like portion increases, and the R diminishes, until finally the S alone remains (fig. 2).

This graded increase in the amplitude of  $S_3$  in the extrasystole depends upon increase in distance of the point of stimulation from the anterior right ventricle, and may be presumed to be the result of increasing delay in the excitation of the anterior right ventricle with respect to the posterior left ventricle. When the point of stimulation is at the anterior septum it must be presumed that the anterior right ventricle begins to discharge before the posterior left ventricle, and that activation of the anterior right ventricle is completed before that of the posterior left ventricle.

When an S wave is found in extrasystoles elicited from points to the left of the septum, the anterior right ventricle must still begin to discharge in advance of the posterior left ventricle, giving the initial R wave. The posterior left ventricle is, however, excited relatively sooner as the point of stimulation is moved to the left, terminating R sooner, and decreasing its amplitude progressively. Excitation of the left ventricle is completed while some of the anterior right ventricle remains to be discharged, giving rise to the downstroke of S, and finally, discharge of regions in the right ventricle terminates S and restores isoelectricity.

This analysis of S in the extrasystole is identical with the explanation given for S in the normal complex.  $S_3$  in the extrasystole should therefore respond as does the normal  $S_3$  to potassium application and to heat and cold. Figure 1 B and D show that this is the case. Figure 1, B2, shows the abolition of S in lead III of the extrasystole by the application of M/5 KCl to the posterior surface of the left

ventricle. In figure 1, B3, the downstroke of S is extended, and the upstroke abolished by application of KCl to the anterior surface of the right ventricle. The S complex in lead III of the extrasystole is also decreased by heating the anterior surface of the right ventricle and cooling the posterior surface of the left ventricle (fig. 1, D2) and increased by heating the posterior surface of the left ventricle and cooling the anterior surface of the right ventricle (fig. 1, D3).

For the development of an S wave in lead III it is necessary that the posterior levocardiogram should have attained its maximum development before the

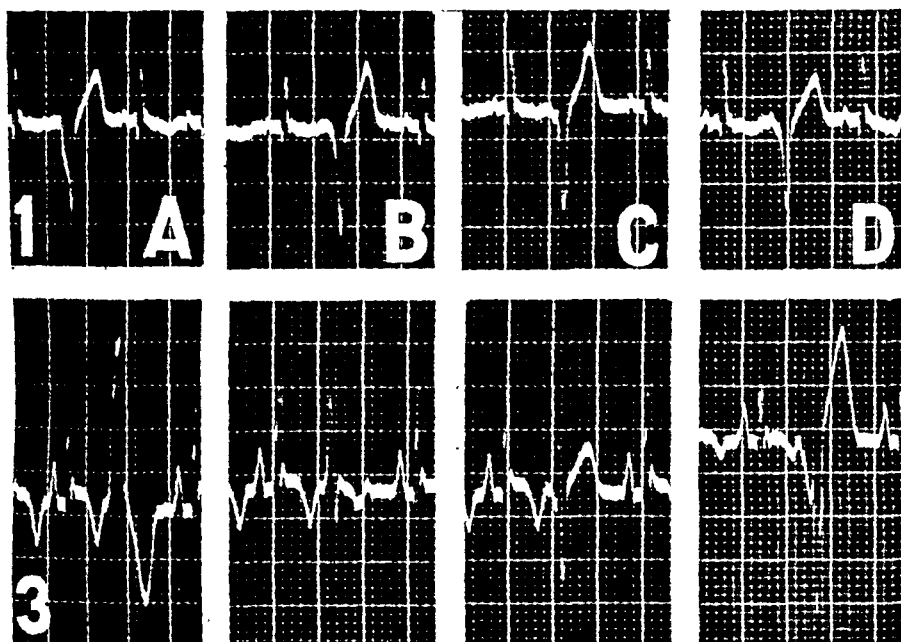


Fig. 2. 9/26/41. Dog, 12 kgm. Leads I and III. A. Extrasystoles elicited by electrical stimulation of a point on the anterior septum midway between apex and base. The initial deflection is downward in lead I and upward in lead III. B. Extrasystoles from a point on the left ventricle 0.75 cm. to the left of the anterior septum. In lead I the complex is essentially unchanged. In lead III the R complex is of diminished amplitude, and an S appears. C. Extrasystoles from a point 1.5 cm. from anterior septum. Lead I is unchanged. Decline in amplitude of  $R_s$  and the increase in  $S_s$ . D. Extrasystoles from the center of the left ventricle. Lead I is unchanged. In lead III R has disappeared and the amplitude of S is maximum.

anterior dextrocardiogram has done so. The preponderance of posterior levocardiogram will then cause a deviation below the isoelectric line (i.e., the downstroke of S). When finally the right ventricle is fully activated, isopotentiality is restored and the upstroke of S described. This postulated retardation in final activation of portions of anterior right ventricle is consistent with reports (5) that a region of the right ventricle over the conus is the last portion of the ventricular surfaces to receive its excitatory stimulus.

An  $S_1$  was found in only one experiment in this series. In that experiment its amplitude was increased by warming the anterior surface of the left ventricle and decreased by warming the posterior surface of the right ventricle. In one other

experiment it was possible to elicit an extrasystole showing an  $S_1$  by stimulation of a point on the posterior surface of the left ventricle.  $S_1$  in this extrasystole was abolished by application of KCl to the anterior surface of the left ventricle. These two observations are consistent with the theory expressed in the introduction regarding the factors concerned with the formation of  $S_1$ .

#### CONCLUSIONS

1. The downstroke of  $S_3$  develops with the complete activation of the posterior surface of the left ventricle while a portion of the anterior surface of the right ventricle is not yet active.

2. The upstroke of  $S_3$  occurs when the remainder of the anterior surface of the right ventricle becomes active and restores isopotentiality.

3. The same sequence of ventricular excitation explains the presence of an  $S_3$  in the ventricular extrasystole.

4.  $S_1$  probably arises from a similar sequence of excitation in the anterior left and posterior right ventricles.

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# SPECIFICITY IN THE RENIN-HYPERTENSINOGEN REACTION

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The volume of experimental evidence which has accumulated within the last few years (Goldblatt et al., 1934; Dicker, 1937; Goldblatt, 1937; Houssay and Fasciolo, 1937; Page, 1939, and others) has established beyond peradventure that renal hypertension—at least in the experimental animal—is causally related to some substance which reaches the general circulation through the venous drainage of the kidneys. This pressor influence of the venous blood draining from chronically or acutely ischemic kidneys, particularly well demonstrated in acute kidney graft experiments by Houssay and Fasciolo (1937), has been generally attributed to an involvement of renin, first found and studied in kidney extracts by Tigerstedt (1897) and Tigerstedt and Bergmann (1898). The work of Braun-Menendez et al. (1939, 1940), Muñoz et al. (1939, 1940) and Page and Helmer (1940a) indicates that this pressor effect of venous blood from the ischemic kidney is not due to a direct action of the renin on the blood vessels. The Buenos Aires group believes the pressor effect is due to “hypertensin”, a product of the interaction of the enzyme renin with a substrate (blood globulins, “hypertensinogen”). Hypertensin is in all probability the substance “angiotonin” of Page and Helmer (1940a). But while these authors spoke of an “enzyme like” nature of renin some time ago (Kohlsteadt, Helmer and Page, 1938) they are not as yet convinced of the verity of the enzyme-substrate mechanism renin-hypertensinogen-hypertensin and maintain that angiotonin (hypertensin) causes no vasoconstriction or pressor action until it is further modified by “angiotonin activator” to give “activated angiotonin” the ultimate vaso-constrictor substance (Page and Helmer, 1940b). The renin activator (hypertensinogen) is reported (Page, McSwain, Knapp and Andrus, 1941) as having its origin in the liver.

The enzymatic action of renin on blood globulins (hypertensinogen) with the resultant formation of hypertensin takes place not only in vivo but also in vitro if renin is incubated with blood plasma at 37°C (Braun-Menendez et al., 1940). The hypertensive substance thus formed in vitro may be very readily determined quantitatively by its action on the blood pressure of a test animal. This in vitro technique provides a convenient and sensitive quantitative method for the estimation of renin in the tissue or solution in question. But the renin and plasma used in such in vitro or in vivo tests for renin cannot be chosen at random, for it has been found that while renin from one mammal reacts with the blood globulins of another to give hypertensin this same renin may fail to react either in vivo or in vitro with the blood globulins of some other mammal; thus, renin prepared from the pig's kidney was found to produce hypertensin when mixed

with plasma of pig, ox, horse and dog but failed to produce hypertensin when mixed with human plasma. Yet renin prepared from human kidney produced hypertensin when mixed with plasma from each of the mammals mentioned (Battro et al., 1940; Fasciolo et al., 1940). It would appear then that there is something of the nature of a specificity in the renin-hypertensinogen reaction. Just why preparations from two different species of the same class, e.g., mammalia-pig renin and human plasma—exhibit renin specificity whereas similar preparations from two others may fail to exhibit such specificity, e.g., dolphin renin and dog plasma (Eichelberger et al., 1940)—is difficult to explain. There may of course be some correlation between the occurrence of renin specificity and the taxonomic position of the various forms but until much more data is at hand no very inclusive conclusions on this point can be reached. It was with the hope of uncovering additional information concerning this specificity and some related problems that the study herein reported was undertaken.

**PREPARATIONS AND PROCEDURES.** The kidney extracts, i.e., the renin solutions, and the plasma (hypertensinogen) solutions used in these experiments were prepared as described earlier (Braun-Menendez et al., 1940). Preparations from the dog, pig, ox, sheep, duck (*Brevortia tyrannus* Cuvier), chicken, toad (*Bufo arenarum* Hensel), fish (elasmobranchii), and man were employed.

*In vivo tests.* One series of tests to determine whether the various renins would react with plasma from the different sources was carried out by the *in vivo* method, viz., the direct intravenous injection of the kidney extract into an anesthetized dog. Except for the control injection of 10 cc. of physiological solution, each injection of 10 cc. contained the extract from 5 grams of kidney. The injection of chicken kidney extract failed to alter the blood pressure appreciably in the test animal. A similar injection of pig renin, however, induced the gradual rise in blood pressure typical of renin. Extract from the toad kidney injected into a fresh test animal failed to produce any alteration in the blood pressure but the injection of extract from sheep kidney resulted in a rise in pressure despite an initial drop which no doubt was occasioned by the presence of some toxic substance.

The fish kidney extracts were obtained from kidneys of 12 recently caught young shark of about 15 kgm. each. Renin has been found in dolphin kidneys hours after their removal and transportation over great distances (Eichelberger et al., 1940). Moreover, the proximity of our laboratory to the source of our material caught by net for these purposes a short time previously and brought directly to the laboratory in the winter season would seem to provide adequate assurance of the freshness of the tissue. Because the kidneys of these elasmobranch fish are to some degree divisible into two parts, one of which is predominantly tubular in make-up, and the other predominantly glomerular in structure, a separate extract was prepared from each of these two portions of the kidneys. It was hoped that if renin be present in these kidneys it might be demonstrable in different concentrations in the tubular or the glomerular portions. But it was found that while the reaction to the injection of the extracts from the tubular and the glomerular portions were not exactly identical

in every detail, neither of the extracts elicited any response in the blood pressure of the test dog which could be interpreted as a pressor reaction to renin. The results of the intravenous injections of the various kidney extracts on the blood pressure of the test dog are included in table 1.

In addition to these *in vivo* experiments in which the dog was used as test animal, further *in vivo* tests were carried out in which graphic tracings of blood pressure were made from the duck, chicken (urethane anesthesia), snake (*Ophis merremii* Wagler, *Xenodon merremii*) and toad (anesthetized by ether or bulbar

TABLE 1  
*In vivo tests*

SOURCE OF RENIN (KIDNEY EXTRACT)	RECIPIENT OF RENIN INJECTION	BLOOD PRESSURE RESPONSE IN RECIPIENT
Toad.....	Dog	No change
Fish (elasmobranch)		
Glomerular portion.....	Dog	No change
Tubular portion.....	Dog	No change
Sheep.....	Dog	Increase
Chicken.....	Dog	No change
Pig.....	Dog	Increase
Toad	Snake Chicken Duck Toad	No change
Fish		
Pig		
Sheep		
Dog		
Human		
Chicken.....	Chicken	Increase
	Duck	Increase
	Toad	No change
	Snake	No change
Pig.....	Human	No change*
Dolphin.....	Dog	Increase†

\* Turnoff, D. and L. G. Rowntree, 1941; Fasciolo et al., 1940; Battro et al., 1940.

† Eichelberger, L., L. Leiter, E. M. K. Geiling, 1940.

pitling) to determine the effect, if any, of intravenous injection of renin preparations of known potency from chicken, fish and toad. None of the mammalian renin preparations or the poikilotherm kidney extracts elicited any pressor response in the duck, chicken, snake or toad. The chicken kidney extract, however, elicited a well pronounced pressor reaction when injected into the chicken as shown in figure 1C, and a somewhat smaller pressor response in the duck but none in the snake or toad.

The fact that chicken renin injected into a chicken causes an increase of blood pressure justifies the conclusion that the chicken possesses hypertensinogen and



forms hypertensin. This, taken with the finding that chicken renin fails to elicit any pressor action in the mammal and that mammalian renin fails to elicit any pressor response in the chicken, indicates the existence of a renin specificity. But in attempting to explain the failure of the poikilotherms to respond to any of the renin injections there is, in addition to the possibility of renin specificity, the factor of body temperature to be taken into account. The low body temperature of itself might conceivably stop the interaction of renin and hypertensinogen even though there were no renin specificity.

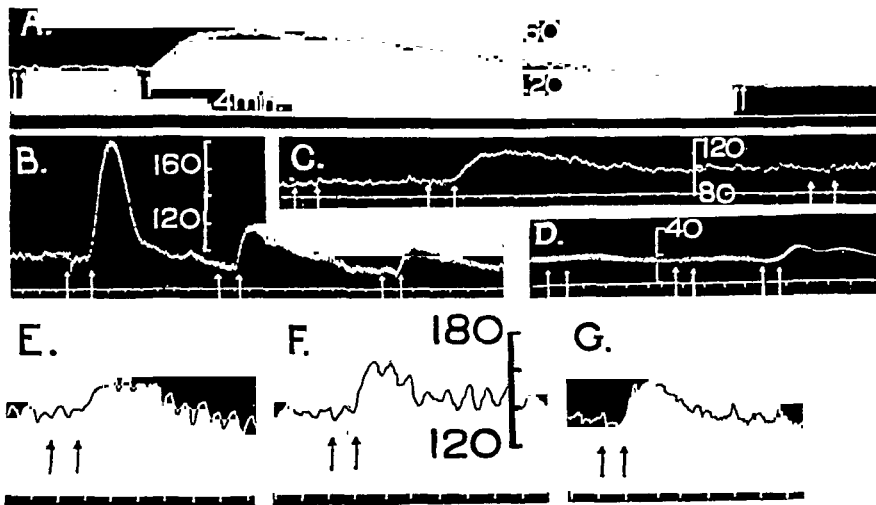


Fig. 1. A. Persistent pressor action of hypertensin in the toad. Arrow couplets mark (1) injection (abdominal view) of 1 cc. Ringer solution; (2) 0.5 cc. hypertensin with 0.5 cc. Ringer; (3) repetition of (2). Note tachyphylaxis. Scale in millimeters Hg.

B. Quantitative response in chicken to hypertensin injection and absence of tachyphylaxis. Arrow couplets mark successive injections of 1.5, 0.5, 0.25 units of hypertensin in equal volumes. Time in minutes.

C. Pressor response in chicken to chicken renin and tachyphylaxis to renin. Arrow couplets mark injection of (1) Ringer solution; (2) 1 cc. chicken renin; (3) repetition of (2).

D. First couplet, injection of 1 cc. of *in vitro* preparation from toad kidney extract and toad plasma (control no incubation); second, injection of 1 cc. of *in vitro* preparation after incubation at 37°C. Third, injection of 0.5 cc. hypertensin and 0.5 cc. Ringer solution. Note the double optima frequently observed in these preparations.

E, F, G. Effect of the first, sixth and eleventh injections respectively of 0.5 cc. of hypertensin into the chicken at 15 minute intervals; blood pressure in millimeters Hg, time in minutes.

In order to diminish the possibility that such a temperature factor might be operating, a number of blood pressure tracings were obtained from toads whose body temperature had previously been raised to 27°C in a water bath and maintained at this temperature throughout the test by a warming plate. But the effects recorded from these warmed toads were essentially the same as those obtained from the unwarmed animals. At temperatures higher than 27° the toads usually succumbed early in the experiment. The data from this series of tests are included in table 2.

*In vitro tests.* The *in vitro* method of preparing hypertensin is particularly advantageous in the study of specificity, not only because of its convenience and the possibility of completely eliminating the factor of low body temperature in dealing with poikilotherms but also because of its greater sensitivity, it being possible to detect renin in quantities 50 to 100 times smaller than by the direct injection method (Muñoz et al., 1940; Leloir et al., 1940). The use of the *in vitro* method should therefore diminish the possibility that a failure of a renin-hypertensinogen

TABLE 2  
*In vitro tests*

KIDNEY EXTRACT (RENIN)	PLASMA (HYPER- TENSINOGEN)	BLOOD PRESSURE REACTION IN DOG	KIDNEY EXTRACT (RENIN)	PLASMA (HYPER- TENSINOGEN)	BLOOD PRESSURE REACTION IN DOG
Toad	Toad Fish Chicken Dog Ox	No change	Pig	Chicken Toad Horse* Pig* Ox* Human* Dog*	No change No change Increase Increase Increase No change Increase
Fish Tubular and glomerular portions	Fish Toad Chicken Dog Ox	No change	Sheep	Chicken Ox† Sheep Human† Dog†	No change Increase Increase No change Increase
Chicken	Chicken Toad Dog Ox Fish	Increase No change	Human	Chicken Dog* Ox* Horse* Pig* Human*	No change Increase
Ox	Ox† Dog† Human† Chicken	Increase Increase No change No change			

\* Fasciolo et al., 1940.

† Braun-Menendez (personal communication).

reaction might be due to a weak concentration of renin in any of the kidneys employed.

A series of experiments using the *in vitro* method was therefore carried out in which the kidney extracts from the various sources were incubated at 37° with the different blood plasmas and the presence of hypertensin in the final preparations tested for by their injection into an anesthetized dog. The blood pressure response to each of these injections is indicated in table 2. Chicken kidney extract produced hypertensin only when reacting with chicken and duck plasmas

(fig. 1B). But none of the renins or kidney extracts of other animals studied reacted positively with chicken plasma. Toad kidney extract not only failed to react with plasma of any other animal but, what is perhaps of greater interest, it failed to react with its own plasma.

The absence of any pressor action in the dog on the injection of toad kidney extract might (taken by itself) be interpreted as due to a specificity of the toad renin just as is the case with the chicken renin; but before such an explanation can be accepted one must be certain that the injected extract contains renin. At present there is no absolutely reliable and universal test for the presence of renin in kidney extracts; but the *in vitro* method of preparation of hypertensin from the kidney extract and blood plasma from the same animal should, since it eliminates the possibility of non reactions arising from the factors of low body temperature and specificity between different animals, provide a very close approximation of such a test. If then toad kidney extract does contain renin, it would be reasonable to assume that it should react with toad plasma to give hypertensin which when injected into the dog as test animal should elicit a typical pressor response. Yet in none of the many attempts to produce hypertensin from toad kidney extract and toad plasma by the *in vitro* method was there any indication of hypertensin in the final preparation. The most probable explanation for such failure of hypertensin formation would seem to be that toad kidney extract does not contain renin. Yet one must admit of other possible, though perhaps less likely, explanations, e.g., an absence of hypertensinogen; the presence in toad blood of some substance which prevents the interaction of renin with the hypertensin precursor; or an exceedingly active hypertensinase which destroys hypertensin before it has had time to become effective on the blood vessel walls. In addition to these there is the further hypothetical possibility that the toad renin—if there be such—and the blood globulins (hypertensinogen) have a very sharp mutual specificity and that the product of their interaction is a substance somewhat different from hypertensin in that it is possessed of a specificity for the smooth muscle of the toad vascular system and fails to induce any pressor influence in the dog. The validity of the last two of these hypothetical possibilities was put to experimental test: that one involving an exceedingly rapid destructive action of toad hypertensinase has been excluded as a possibility by the evidence from experiments on toad hypertensinase presented farther along in this report; and that one based on a possibly sharp specificity of some unknown pressor substance for toad smooth muscle was investigated by a number of experiments in which the preparation obtained from *in vitro* incubation of toad kidney extract with toad plasma was injected intravenously into toads at normal body temperature and at temperature artificially elevated to 27°C. No alteration in the blood pressure occurred—results which justify the conclusion that there is neither hypertensin nor any other pressor substance possessed of some peculiar specificity for toad smooth muscle formed by the interaction of toad kidney extract and toad plasma.

While it cannot be categorically denied that either hypertensinogen or renin or both may be lacking in the toad or that toad blood contains some substance which prevents the interaction of renin with hypertensinogen, perhaps the most probable

explanation for the absence of hypertensin formation in the toad is an absence of renin. This interpretation finds support in the experimental observation that artificially induced ischemia of frog kidney does not influence the frog's blood pressure (Vogt, 1941).

*The persistent action of hypertensin in poikilotherms.* In mammals such as the dog the typical pressor response to a single intravenous injection of a standard solution of hypertensin is rapid in its onset, the gradient sharp and the duration relatively short. The pressor response to similar doses injected into the chicken was found to be practically identical with that of the mammal, not only in the rapidity of onset but also in its gradient, duration, and its striking quantitative nature. The latter feature is especially well illustrated in figure 1B. Such distinctly proportional responses would justify the use of the chicken as a test and assay preparation for the quantitative determination of hypertensin.

In the toad (fig. 1A) and snake, however, the pressor response to hypertensin was different; the gradient was low, there was a very pronounced persistence of the pressor effect and occasionally—especially with the larger doses—the curves presented two optima (fig. 1D). The slow circulatory rate and the sluggish response of smooth muscle at the lower body temperature of these poikilotherms no doubt contributed to the occurrence of these differences but such cannot be the complete explanation; the persistent pressor effect might very well be due to a slow removal of hypertensin from the blood of the poikilotherms. In the mammal hypertensin is destroyed or its action nullified by an enzyme substance hypertensinase (Muñoz et al., 1940) so that the relatively short duration of the pressor effect of a single injection of hypertensin into a mammal or chicken is in all probability due to a rapid removal of hypertensin from the blood largely by a rapid destructive action of hypertensinase. On the other hand, the prolonged and persistent pressor action of a similar dose of hypertensin in the toad or snake, as described above, might well be due either to a complete absence of hypertensinase in these forms or, if it be present, to a sharp retardation in its destructive action as a consequence of the low body temperature of these animals. Such retardation together with the sluggish circulation and smooth muscle response at these temperatures could well account for the persistent pressor effect of hypertensin.

Hypertensinase is present in extracts from mammalian tissues such as liver and spleen and it is also found in blood plasma and hemolyzed red blood cells (Muñoz et al., 1940). If it is present in the toad it should be readily demonstrable in some of these same tissues. Tests for the presence of hypertensinase in the toad were made on toad plasma: 1 cc. of plasma was added to 9 cc. of a buffered solution of hypertensin, made up of 1 cc. of hypertensin standard, 0.5 cc. phosphate buffer, 0.2 cc. merthiolate, and 7.3 cc. of water, and the mixture incubated at 37°C for 2 hours. It was then boiled for 10 minutes. A control solution was prepared by mixing 1 cc. of toad plasma with 9 cc. of the buffered hypertensin solution after which it was immediately boiled for 10 minutes to prevent any destruction of hypertensin by the hypertensinase if such should be present. In the same manner solutions were prepared using 1 cc. of a solution of hemolyzed

red blood cells (1 cc. of centrifuged red blood cells hemolyzed in 9 cc. of distilled water) in place of the plasma. After cooling these incubated test and the non-incubated control solutions, they were injected into the dog as test animal. The injection of control solutions of both the plasma and red blood cells evoked a rise in blood pressure characteristic of 1 cc. of hypertensin standard; the incubated test solutions evoked no change whatsoever. It is to be concluded, therefore, that toad plasma and toad red blood cells do contain the hypertensin destroying enzyme hypertensinase.

This demonstration of the existence of hypertensinase in the blood of the toad dismisses the possibility that the prolonged and persistent action of hypertensin injected into the toad might be due to the absence of this hypertensin destroying substance. There remains the question of whether the persistence of the pressor response to hypertensin in poikilotherms might not be due to a slowed destruction of the hypertensin by hypertensinase at the lower body temperature of these animals. In order to answer this query, the experiments on toad plasma and hemolyzed red blood cells described above were repeated except that the incubation of the test solutions was carried out at 18°C rather than at 37.5°C. The injection of these test solutions evoked a blood pressure rise practically identical with that of their non-incubated controls. It is to be concluded, therefore, that hypertensinase of toad plasma and red blood cells is almost completely inactivated by temperatures as low as 18°C and that the persistence of the hypertensin pressor effect in poikilotherms is due for the most part to the inactivation of hypertensinase by the low body temperature which in these tests approximated 15°C.

The pronounced effect of temperature at this lower level seemed to warrant a study of the effects of temperature on the hypertensin-hypertensinase reaction over a wider range. The results of such additional temperature studies are summarized graphically in figure 2, where temperature has been plotted against the amount of hypertensin (in terms of 1 unit standard) remaining in the solution in question after 2 hours of incubation with hypertensinase at the various temperatures. The destructive action of hypertensinase bears an inverse relation to the curves in the figure so that where there has been little or no hypertensinase activity the amount of hypertensin remaining, i.e., as indicated by the pressor response, is high. The curves show that for temperatures below 15°C, the hypertensinase of both the dog and the toad is completely inactivated and that for temperatures above 20°C the activity is sharply increased up to an optimum of about 40°C. The finding that the toad hypertensinase-temperature activity curve is essentially similar to that for dog hypertensinase dismisses the possibility mentioned earlier in this report, that the absence of any pressor response in the toad to the injection of toad kidney extract could be due to some peculiarly rapid action of toad hypertensinase. Since the curves for hypertensinase from bovine and human plasmas shown in this figure summarize a limited number of tests they are to be regarded only as tentative and await further verification.

*Tachyphylaxis.* It is generally agreed that renin induces tachyphylaxis, i.e., administration of renin renders that animal less responsive to successive doses.

There is however a lack of agreement concerning the question of whether hypertensin induces a similar tachyphylaxis. It has been reported that hypertensin (angiotonin) does induce tachyphylaxis (Herrick et al., 1941) and there is offered an explanation—on the basis of an exhaustion of “angiotonin activator” and a production of an “angiotonin inhibitor” (Page and Helmer, 1940b). On the other hand the fact that anesthetized test dogs, such as are used in the assay of hypertensin preparations, consistently show no significant diminution in their pressor response to hypertensin injections even after as many as 30 and 40 successive administrations, would seem to constitute abundant evidence that hypertensin does not produce tachyphylaxis in the dog to any significant degree.

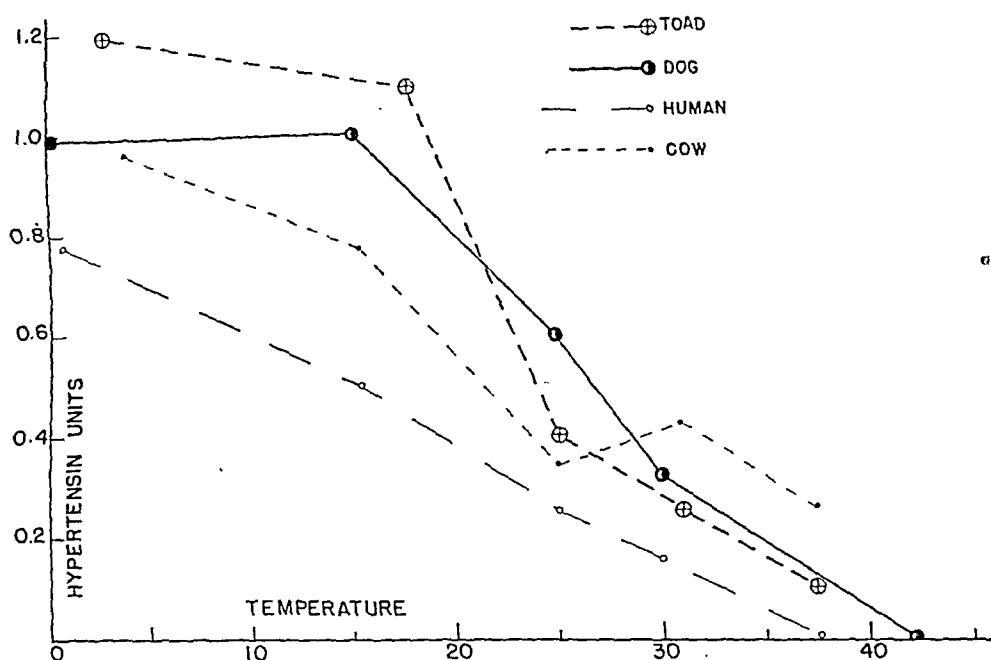


Fig. 2. Hypertensinase activity as determined from the amount (units) of hypertensin left in the solution after 2 hours incubation at the various temperatures with hypertensinase.

In the chicken, too, there is a demonstrable absence of hypertensin tachyphylaxis. This is shown in figure 1E, F, G, where the blood pressure response to the eleventh injection of 1 cc. of hypertensin standard was actually a trifle greater than was the response to the first injection. On the other hand, the pressor response to successive injections of renin is quite different; injections of renin into the chicken induce a pronounced tachyphylactic state as is shown in figure 1C, where the second injection of chicken renin into a chicken failed to elicit any pressor response although the response to the first injection was well marked. In this tachyphylactic reaction to renin, the chicken is in agreement with the renin tachyphylaxis which occurs in the dog.

Turning now to the question of tachyphylaxis in poikilotherms, the evidence from the perfusion of the L wen-Trendelenberg preparation (Braun-Menendez et al., 1940) would seem to justify the interpretation that hypertensin does not

induce tachyphylaxis in the toad. However in view of the possibility that the conditions obtaining in perfusion preparations and in the intact circulated animal might not be directly comparable it was thought advisable to investigate the question of hypertensin tachyphylaxis in the toad by direct recording of the blood pressure response to hypertensin injected into the abdominal vein of pithed or ether anesthetized toads. From these experiments it was found, without exception, that the pressor effect of hypertensin diminished with each succeeding administration until eventually no pressor response could be elicited. In figure 1A is shown the pressor response in the toad to successive injections of 0.5 cc. of hypertensin into the abdominal vein. The first resulted in a pronounced rise in pressure; the second injection was without effect and illustrates a rapid onset of hypertensin tachyphylaxis. The records of blood pressure taken from the ascending aorta of the snake likewise show the phenomenon of tachyphylaxis to standard hypertensin. The occurrence of hypertensin tachyphylaxis in the poikilotherms tested may be closely related to the lack of a complete renin-hypertensinogen-hypertensin system in these forms; or perhaps the persistent action of the injected hypertensin as a result of retarded hypertensinase activity is a contributing factor. In any case this tachyphylaxis to hypertensin in the poikilotherms is more pronounced with the larger doses. Why hypertensin tachyphylaxis should be absent in the L wen-Trendelenberg preparation on the one hand and present in the normal blood circulated toad on the other is difficult to explain adequately. Conceivably a number of the differences between conditions obtaining in the two techniques might be involved, e.g., a continuous washing of the blood vessels by fresh perfusate in the L wen-Trendelenberg preparation as compared with an uninterrupted re-circulation of blood in the circulated animal may contribute some essential factor to the occurrence of hypertensin tachyphylaxis in the toad.

#### SUMMARY

The question of the specificity of renin was investigated by the *in vivo* (direct intravenous injection of kidney extract into a test animal) and the *in vitro* (intravenous injection of alcoholic extract of kidney extract incubated with hypertensinogen) methods.

Kidney extracts from the sheep, ox, dog, pig, chicken, shark, toad and plasma preparations (hypertensinogen) from ox, dog, chicken, shark, toad and man were employed.

The tests of the various kidney extracts and hypertensin solutions prepared by the *in vitro* method were carried out by continuous arterial blood pressure recordings on dogs, chickens, ducks, toads and snakes.

The specificity of renin, which is manifest by a failure of the renin to react with hypertensinogen from another source, was demonstrated in a number of vertebrates of mammalian, avian, amphibian, reptilian and fish forms. This specificity, which has been found to exist between the classes studied, has also been found between some individuals within a class, e.g., mammals. Mammalian renin such as from the pig does not react positively with hypertensinogen from

the chicken; duck, toad, snake or shark, and chicken renin does not react positively with hypertensinogen from any of the mammals studied. Toad kidney extract injected into a toad at normal or artificially elevated body temperature fails to elicit any pressor response. In vitro preparations from the incubation of toad kidney extract with toad plasma fails to elicit any pressor response when injected either into the toad or dog. Hypertensin is not formed in the toad, and in all probability in none of the poikilotherms, due to an absence of some essential part, or all, of the renin-hypertensinogen-hypertensin system. The most likely missing link would appear to be renin.

Hypertensinase, the hypertensin destroying enzyme, is present in toad plasma, but it is practically completely inactivated by temperatures below 15°C. Its optimum temperature is 40°C. The temperature-activity curve for hypertensinase of dog blood conforms essentially with that of toad hypertensinase.

The persistence of the pressor effect of a single injection of hypertensin into poikilotherms such as the toad and snake is due largely to the long life of hypertensin in the circulation of these forms. This prolongation results from the retardation of the destructive action of hypertensinase by the low body temperature of these animals. In contrast to the chicken and mammal, the toad and snake are rapidly rendered tachyphylactic to hypertensin by hypertensin injections into the blood stream.

The chicken shows no significant tachyphylaxis to injections of hypertensin, but it does exhibit a pronounced tachyphylactic response to renin. Its behavior in this respect is similar to that of the dog which commonly shows no tachyphylaxis after as many as 40 injections of hypertensin.

The quantitative pressor response of the chicken to hypertensin is such as to warrant the use of the chicken in assay procedures.

It is a pleasure for me to express my appreciation for the kindly personal interest and help afforded me by Professor Houssay and his Staff at the Instituto de Fisiologia, Buenos Aires, during my stay there, where this work was carried out.

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# MUSCLE POTENTIALS ACCOMPANYING A SINGLE VOLITIONAL TWITCH

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Gilson and Mills (1940) have demonstrated that a discrete volitional effort, if sufficiently slight and brief, may result in a single response of the motor unit near the recording electrode. They also state that: "Somewhat quicker movements cause the appearance of double spikes due either to repetitive discharge of the original unit, or to the introduction of potentials from a second nearby motor unit which had previously been inactive."

The present authors have confirmed the observation that slight movements may involve a single discharge of a single motor unit. The response accompanying somewhat stronger twitches is the subject of the present paper.

**METHODS.** Needle electrodes lacquered to the tips were used to lead off from various muscles of the arm or hand. The needles were thrust through the skin over the muscle from 3 to 20 mm. apart, sharp localization not being necessary. Action potentials were led off through a four-stage capacity-coupled amplifier having a time constant of 0.16 sec., and recorded from a W. E. 326C Braun tube. To facilitate recording, the early portion of the action potential was used to key off the sweep circuit.

Electromyograms during brief volitional twitches were studied in six adult subjects in m. abductor pollicis brevis, m. flexor sublimis digitorum, m. biceps brachii, m. flexor carpi radialis, and m. extensor digitorum communis.

**RESULTS.** The simplest and smallest response observed was a single discharge of a single motor unit (fig. 1A and B), identified by its constant form and its appearance in records of sustained contraction. However, in spite of the subject's effort to repeat the same type and degree of twitch, the responding unit was not always the same. For example, in one experiment any one of three different units might respond with a single discharge.

Slightly stronger efforts were found to involve, not double or repetitive discharges of a single unit, but either 1, overlapping discharges of two or more units, or 2, serial discharges of two or more units, or 3, a combination of both 1 and 2.

Strong effort likewise gave rise to these types of responses. Single discharges, ten or more times the amplitude of a single motor unit potential, were observed. On the other hand, a response might be initiated by a single motor unit, followed by a complex of large spikes. The twitch response was never observed to consist of a burst of discharges from a single motor unit. Nonetheless a unit might repeat once or more in a response involving other units (fig. 2, N and O).

Double discharges of a single unit were occasionally seen, but only with a latency of the same time order (about 100 msec.) as that of repetitive discharges during a sustained contraction (fig. 2P). With an effort to prolong a slight twitch

it was found possible to repeat such double responses time after time. However, double discharges of very short interval were never seen in some two hundred cathode-ray oscillograms.

In one subject, 314 twitch electromyograms at varying degrees of tension were recorded with the ink-writing oscillograph. Only 10 of these appeared to be

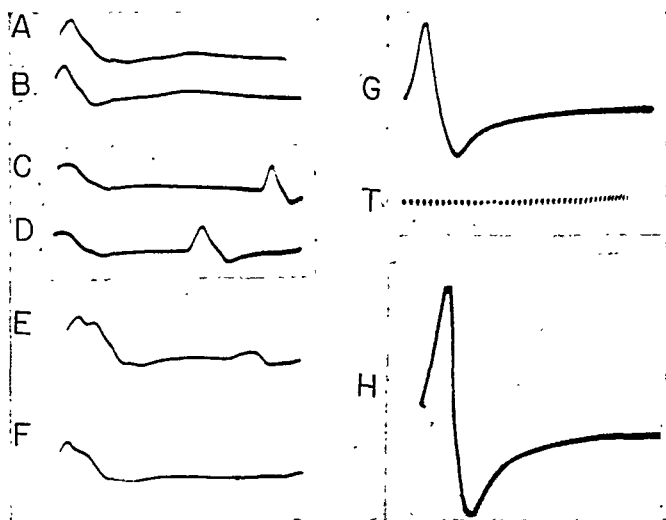


Fig. 1. Electromyograms from abductor pollicis, brief volitional twitches, subject J. T. Rising phase of first spike used to key off sweep circuit. Motor units  $x$  and  $y$  were low threshold units in sustained contraction.

(a, b) Single unit  $x$ . (c, d) Single unit  $y$  followed by  $x$ . (e, f) Overlapping  $x$  and  $y$ , third unit in (e). (g, h) Large spikes of variable amplitude, attributed to several motor units firing synchronously. (t) Time, msec.

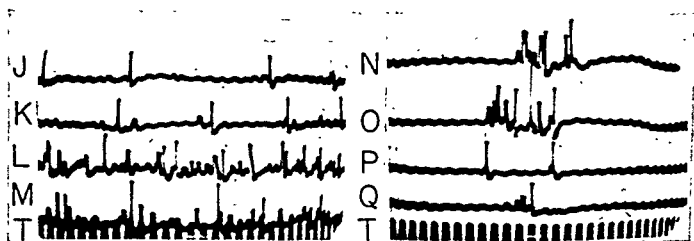


Fig. 2. Electromyograms from abductor pollicis, subject R. O.

(j, k, l, m) Sustained contraction. (n, o) Moderate twitches. Some units repeat. (p) Double response of single unit. Interval about 90 msec. (q) Three unit response. (t) Time, 60 per second.

double discharges of a single unit. Of these, 7 were 85 msec. or longer in interval, and the other 3 were doubtful cases. In 21 complex discharges where a unit was seen to repeat, the interval varied from 40 to 140 msec. The most frequent type of discharge was a combination of serial and overlapping types with no apparent repetition (107 cases). Discrete serial discharges (70) were more frequent than the overlapping type (53). The remaining 53 were single spikes.

DISCUSSION. From the observations given above it would seem that the type of double response observed by Gilson and Mills (1940, 1941) is probably due to the asynchronous discharge of two motor units rather than the repetitive discharge of a single unit. At slow sweep speeds it may be difficult to discriminate between two units whose form is readily differentiated at higher sweep speeds. Such two-unit responses with intervals of from 4 to 100 msec. have been repeatedly observed from all subjects used in the present work. Although true double discharges from single units with intervals as short as 10 msec. have been observed in reflex responses of cat muscle by Denny-Brown (1929), Eccles and Hoff (1932), and Adrian and Bronk (1929) the present work does not support the existence of this type of response in the human electromyogram.

For delicate and rapid volitional movements it would seem that the possibility of more or less simultaneous firing of many units once and not more than once would provide greater speed of movement than repetitive firing of single motor units, and thus have obvious utility in such human pursuits as the playing of musical instruments. That a volitional twitch consisting of a high frequency burst from a single motor unit should occur under normal conditions seems improbable from the usual observations on sustained contractions. For example, with increasing effort a low-threshold motor unit which begins to fire at 7 per second may be joined by another unit firing at a low rate when the first unit has reached 10 per second, and by a third when the first unit has reached 13 per second, etc. It is unlikely that in a short twitch a single unit could produce a tetanic burst unless the next-ranking units also entered into the response. That the latter does occur is indicated by the asynchronous responses in which one unit may repeat but other units also take part.

The observation that the same motor unit may be the low threshold unit for both the twitch and sustained type of voluntary contraction suggests that in the case of the arm and hand muscles of man there is no sharp differentiation at the motoneurone level into units of tonic and ballistic function.

#### SUMMARY

It is confirmed that the simplest possible voluntary movement involves a single discharge of a single motor unit. Stronger twitches involve, not repetitive discharges of a single unit, but either 1, the synchronous or overlapping discharge of two or more units, or 2, the serial discharge of two or more units, or 3, a combination of both 1 and 2.

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# THE EFFECT OF ETHER AND STARVATION ON LIVER GLYCOGEN MAINTENANCE AFTER VARIOUS DIETS

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Mirski and his associates (1) in 1938 placed rats on diets high in carbohydrate and protein respectively, and studied the liver glycogen content after subjecting the animals to starvation for varying lengths of time, exercise to the point of exhaustion, or administration of typhoid vaccine, phloridzin, or dinitrophenol. They found that the livers of animals receiving the high carbohydrate diet were richer in glycogen than those of the rats receiving the high protein diet. After 16, 24 and 48 hours' starvation, and after phloridzin, the liver glycogen of the animals fed the high protein diet had diminished much less than that of the rats on the high carbohydrate diet. Shortly after exercise, or after the administration of one of the substances mentioned, there was a notable decrease in liver glycogen in all cases. After a recovery period of 4 to 24 hours, the glycogen of the carbohydrate series was still falling, while that of the high protein group had increased again.

Our experiments were designed to discover which diet would be more favorable in maintaining the liver glycogen of animals subjected to ether anesthesia. In addition, rats were starved for 8 and for 24 hours in order to provide a basis for comparison.

**EXPERIMENTAL.** Male albino or hooded rats weighing between 150 and 300 grams were kept on two types of experimental diets for 5 to 17 days. The high carbohydrate diet consisted of casein 20 per cent, starch 50 per cent, sucrose 20 per cent, and cottonseed oil 10 per cent. The high protein diet consisted of casein 90 per cent and cottonseed oil 10 per cent. To 100 grams of each diet were added 4 grams of inorganic salt mixture (Steenbock), 2 grams of brewers' yeast, and 25 drops of cod liver oil.

Rats on each diet were divided into five groups. Group I was not starved. Group II had food withheld for eight hours. Group III was starved for twenty-four hours. Group IV was not starved and received ether for a period of thirty to forty minutes. Group V had food withheld for eight hours, then was given ether in the same way and finally was allowed to recover but was not fed for the next sixteen hours.

All animals except those in group IV were operated on in the morning. They were given 75 mgm. of pentobarbital sodium per kilogram of body weight. When deep narcosis occurred, the abdomen was opened and two slices of liver each weighing between 0.8 and 2.0 grams were removed and immediately immersed in 2.0 to 4.0 mil. of 30 per cent potassium hydroxide in weighed, stoppered test tubes. The glycogen was isolated and hydrolyzed by the method of Good, Kramer and Somogyi (2). The resulting glucose was determined by the Benedict method (3) adapted to the Klett-Summerson photoelectric colorimeter.

The rats of groups IV and V were placed, four to six at a time, under a large bell jar into which flowed a mixture of oxygen and ether. The rats were kept fully anesthetized for a period of thirty to forty minutes. The rats of group IV were then operated upon as described above. Those in group V, as noted previously, were allowed to recover, but were not fed; 16 hours later they were given pentobarbital sodium and operated upon.

RESULTS. The experimental data are summarized in table 1. The values obtained in groups I and III confirmed the findings of Mirski and his co-workers with respect to the effect of high carbohydrate and high protein diets, and of starvation for 24 hours following these diets, upon the liver glycogen. In group II at the end of 8 hours' fasting, the loss of glycogen from the livers of carbohydrate-fed animals was greater than that of the protein-fed animals, but these differences are not statistically significant. In the non-starved animals (group IV) ether produced a liver glycogen loss of 40 per cent in the carbohydrate-fed

TABLE 1

GROUP	DIET	TREATMENT	NO. OF RATS	GLYCOGEN PER CENT (AS GLUCOSE)
I	Carbohydrate	Not starved	9	4.0 $\pm$ 0.32
I	Protein	Not starved	7	2.5 $\pm$ 0.53
II	Carbohydrate	Starved 8 hours	5	2.7 $\pm$ 0.95
II	Protein	Starved 8 hours	5	1.9 $\pm$ 0.62
III	Carbohydrate	Starved 24 hours	3*	0.23 $\pm$ 0.064
III	Protein	Starved 24 hours	2*	1.04 $\pm$ 0.16
IV	Carbohydrate	Etherized, not starved	5	2.4 $\pm$ 0.94
IV	Protein	Etherized, not starved	4	0.49 $\pm$ 0.22
V	Carbohydrate	Starved 24 hours, ether at 8 hours	6	0.37 $\pm$ 0.12
V	Protein	Starved 24 hours, ether at 8 hours	5	0.48 $\pm$ 0.18

\* Experiments limited to a small number of rats when the results were seen to confirm those of Mirski's similar experiments.

animals and a loss of 80 per cent in the protein-fed animals. However, the absolute losses of glycogen were 1.6 grams percent and 2.0 grams percent respectively. These findings indicate that ether produces glycogenolysis of about the same magnitude regardless of the previous diet. The experiments combining the effects of ether and starvation (group V) produced losses of 91 per cent in the carbohydrate-fed animals and 81 per cent in the protein-fed animals, the resulting glycogen levels being close to minimal. The implications of these results will be discussed later.

DISCUSSION. Mirski (1) explained his results on the basis of accelerated glycconeogenesis from the general body protein in animals on high protein diets after subjection to various procedures designed to deglycogenate the liver, although he notes that these animals probably utilize less carbohydrate than those previously on a high carbohydrate diet.

The level of glycogen in the liver represents a balance between rates of glycogenesis and glycogenolysis. Peters (4) points out that when the supply of car-

bohydrate in the diet is large, it is used as practically the sole source of energy, especially in the working muscles. Glycogenesis and glycogenolysis both proceed at a rapid rate. When food is withheld, less carbohydrate is burned; when, finally, protein breakdown is the principal source of carbohydrate, the latter is burned only for obligatory purposes (metabolism of nervous tissue, mainly). An additional amount of carbohydrate thus formed is stored as glycogen in the liver.

However, protein evidently does not begin to form carbohydrate until deglycogenation of the liver is almost complete, a process requiring 24 to 48 hours in the starved rat, and considerably less time in rats exposed to various deglycogenating agents. On the other hand, when carbohydrate is again made available after starvation, combustion of the newly supplied carbohydrate is minimal and this sugar goes to form liver glycogen; hyperglycemia and sometimes glycosuria occur until carbohydrate metabolism again speeds up. In either case there is a definite lag period prior to the adjustment of the animal's metabolism to its metabolic mixture, whether exogenous or endogenous.

According to this view, our starvation experiments and those of Mirski (1) would indicate minimal carbohydrate oxidation in those animals previously on a high protein diet, while rats of the carbohydrate series continued to burn carbohydrate freely.

Ether anesthesia produced considerable and approximately equal diminution in liver glycogen in animals previously fed carbohydrate and protein. This indicates, as Mirski (1) previously stated, that there is no difference in the glycogen formed or its availability in the animals fed either diet. We have no evidence bearing on the fate of the carbohydrate thus mobilized.

In group V of our experiments the effect of starvation was superimposed upon the effect of ether on the liver glycogen. The character of the previous diet had no demonstrable effect on the liver glycogen levels after 24 hours. If increased glyconeogenesis occurred in either series during the 16 hours subsequent to ether anesthesia, it did not result in an increase in the glycogen level.

Our results and those of Mirski (1) may then be explained by the concept that the carbohydrate content of the metabolic mixture largely determines the extent of the basal carbohydrate oxidation. The level of liver glycogen is modified by exercise, drugs, and the like, and by the lag occurring in the adjustment of carbohydrate utilization to carbohydrate supply, when the metabolic mixture undergoes a considerable change in composition.

#### SUMMARY

1. A series of experiments has been performed to assay the effect of ether anesthesia on the liver glycogen of rats fed high carbohydrate and high protein diets respectively. These experiments indicated that both groups lost about the same quantity of glycogen, although the group on the high carbohydrate diet had higher initial liver glycogen concentrations.

2. The results of experiments by Mirski and his associates on rats starved after receiving high carbohydrate and high protein diets were confirmed.

3. A possible explanation of the results of our experiments and of Mirski's has been presented.

Our thanks are due to Stephen Yohalem, M.D., for his technical assistance.

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# TRANSFER OF WATER ACROSS THE PLACENTA OF THE GUINEA PIG

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Observations on the transfer of sodium across the placenta of the guinea pig, using radioactive sodium as the tracer material, have previously been reported (1). The present study concerns the passage of water from mother to fetus; heavy water (deuterium oxide) has been used as the tracer substance. As with sodium, this study has as its purposes an evaluation of the rate of transmission of water across the placenta at various stages of pregnancy, and a comparison of the growth rate of the fetus and the rate at which water is supplied to the fetus at different periods of gestation.

**METHODS.** The  $D_2O$  has been injected intravenously in sufficient quantity to give a blood concentration for analysis of about 0.6 per cent. Depending upon the weight of the animal, this amounted to from 2 to 5 cc. of 95 per cent  $D_2O$ .

The fetuses were delivered by cesarean section at a known interval of about 10 minutes after injection of the  $D_2O$ . This interval was fixed by the results on rate of equilibration of the fetus with the  $D_2O$  of the maternal blood (fig. 1). A sample of heart's blood was taken from the mother immediately after delivery of the fetuses. The placentae, after removal of excess blood, and the fetuses, were weighed.

The water from the maternal blood and the fetuses was obtained by vacuum distillation to dryness at room temperature and was condensed in tubes surrounded by solid  $CO_2$ . It is essential that the distillation be carried to complete dryness because of differences in the rate of distillation of  $H_2O$  and  $D_2O$ . The water was next purified by passing it over  $CuO$  in a combustion furnace and distilling it from alkaline permanganate and chromium trioxide as described by Keston, Rittenberg and Schoenheimer (2).

The concentration of  $D_2O$  in the samples was determined by a modification (2) of the procedure of Barbour and Hamilton (3). This consists of measuring the falling time of a drop of water of fixed volume through a column of an immiscible fluid of slightly lower density which is maintained at constant temperature. At the suggestion of Dr. David Rittenberg we have used m-fluorotoluene obtained from the Eastman Kodak Company as the immiscible medium and it has been maintained at a temperature of  $19.5^\circ$ . Other details of the procedure, including the micropipet, were as described by Keston, Rittenberg and Schoenheimer (2).

Doctor Rittenberg has checked values obtained in our laboratory to within  $\pm 0.02$  per cent. We have run our samples in duplicate through the stages of purification and determination of falling time and have consistently obtained checks to within  $\pm 0.01$  per cent.

RESULTS. Observations on the change of concentration of DHO, intravenously injected, in the blood with time have previously been given (4). For the present experiments the important results of this investigation are the shape of the time-concentration curve, and the conclusion that the DHO in the blood comes to equilibrium with extravascular fluid in about 7 minutes and that its concentration remains constant for at least 2 hours thereafter.

*Establishment of equilibrium between maternal blood and fetus.* Figure 1 presents data from which it is possible to gain an estimate of the rate at which the fetus comes to equilibrium with DHO in the maternal blood. Similar experiments with radioactive sodium showed that the fetus comes to 80 per cent of equilibrium with the maternal plasma only after about 4 hours (1). In

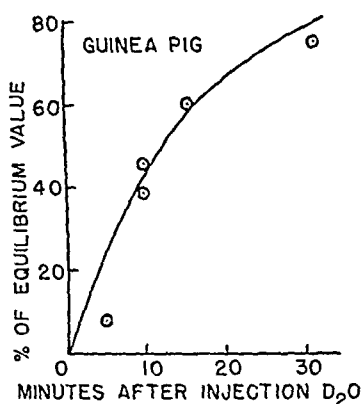


Fig. 1

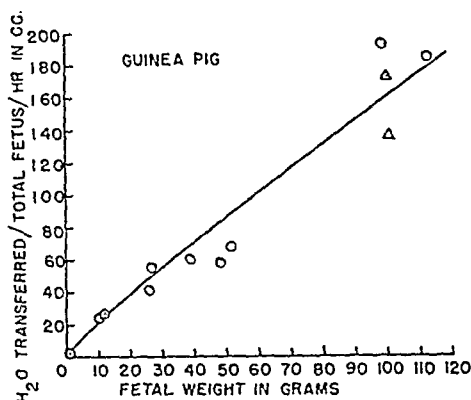


Fig. 2

Fig. 1. Rate of equilibration of fetus with DHO in maternal blood. The ordinates were obtained by dividing the observed concentrations of DHO in the water of the fetus by the corresponding concentrations of DHO (average as explained in text) in the water of the maternal blood and multiplying by 100.

Fig. 2. Variation of rate of transfer of water to fetus with respect to fetal weight. The two points within triangles were obtained from fetuses at term but from a mother of different and considerably smaller (30 per cent) stock than that used for the remainder of the experiments. These two fetuses weighed 70 grams.

sharp contrast to  $Na^{24}$ , DHO in the fetus comes to 80 per cent of equilibrium in approximately half an hour. The period of linear rate of exchange between maternal blood and the fetus is consequently much shorter with DHO than with  $Na^{24}$ . We have chosen, in the experiments reported here, to remove the fetuses at a known interval of about 10 minutes after intravenous injection of  $D_2O$  into the mother. As is evident in figure 1, it appears that the rate of exchange in this interval does not deviate in any important way from linearity.

*Variation of transfer rate per unit weight of placenta with gestation age.* The experimental observations are given in table 1. The only item calling for explanation is the corrected value for DHO in the blood. In order to calculate the rate of passage of water across the placenta, it has been necessary to correct the observed DHO value and to use the average concentration of DHO in the

maternal blood for the ten minutes of the experiment. During the first 5 or 6 minutes after intravenous injection of  $D_2O$ , the concentration in the blood falls to about one-thirteenth of its initial value and the fetus is consequently exposed, during the first half of the experiment, to a concentration of DHO in the maternal blood which is rapidly changing. The average concentration has been obtained from the curve relating concentration of DHO in the maternal blood to time after its intravenous injection (4). The area under the curve up to the delivery time of the fetus was measured and the ordinate of average concentration ob-

TABLE 1

*Values from which the data of figures 2, 3 and 4 have been derived*

Delivery time refers to the time of delivery of the fetus after injection of  $D_2O$  into the mother. Per cent DHO in blood water (corrected) is the average concentration of DHO in the blood up to the delivery time and has been derived as explained in the text. Litter mates are grouped together.

FETAL WEIGHT	DELIVERY TIME	PLACENTAL WEIGHT	TOTAL $H_2O$ OF FETUS	DHO IN FETUS WATER	DHO IN BLOOD WATER	DHO IN BLOOD WATER (CORRECTED)
<i>grams</i>	<i>minutes</i>	<i>grams</i>	<i>cc.</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0.73	10.5	0.33	0.63	0.400	0.460	0.702
0.85	11.6	0.35	0.82	0.398	0.657	1.063
10.8	10.8	2.90	9.5	0.386		0.805
12.0	11.6	2.20	10.4	0.406	0.486	0.788
26.6	11.5	3.50	22.3	0.513		1.090
26.0	12.0	3.50	21.4	0.414	0.670	1.072
39.0	10.0	2.90	31.9	0.330	0.618	1.055
48.0	15.0	3.50	39.5	0.230		0.628
52.0	13.2	3.80	42.0	0.230	0.424	0.656
71.0	10.5	3.70	53.5	0.491		1.100
73.0	9.9	4.60	52.4	0.618	0.660	1.135
98.5	9.7	4.80	67.4	0.487		1.078
112.2	10.1	4.40	79.2	0.414	0.618	1.065

tained by dividing the elapsed time in minutes into the area. The correction factor was then equal to the ratio of the ordinate of average concentration and the ordinate at the time of delivery.

On the assumption that there is no separation of isotopes (1) the quantity of water transferred to the fetus can be directly calculated from the observed concentration of DHO in the fetus water, the corrected concentration of DHO in the water of the maternal blood and the total volume of water of the fetus. If these quantities be designated respectively by  $DHO_{TF}$ ,  $DHO_{MB}$ , and  $H_2O_F$ ,

then the quantity of water transferred to the fetus during the time of the experiment is equal to:

$$\frac{DHO_{T.F.}}{DHO_{M.B.}} \times H_2O_F.$$

The values for the quantity of water transferred to the fetus per hour at various fetal weights is given in figure 2.

Using the values of figure 2 and table 1, the transfer of  $H_2O$  per gram placenta per hour has been obtained by dividing the quantity of water transferred to the fetus by the corresponding placental weight. These values are presented in figure 3. It is evident that about 9 times as much  $H_2O$  is transferred across a

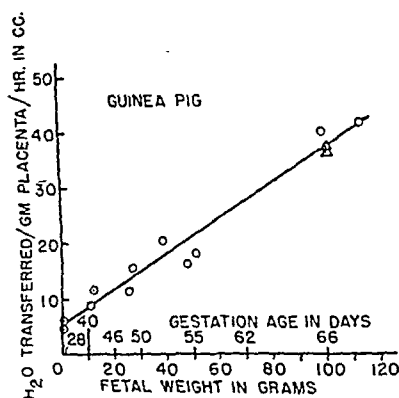


Fig. 3

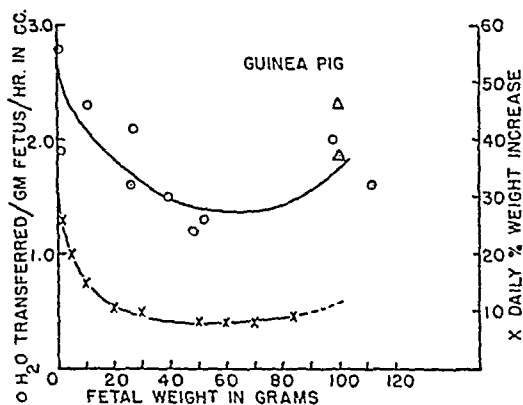


Fig. 4

Fig. 3. Variation of rate of transfer of  $H_2O$  per unit weight of placenta with respect to fetal weight or gestation age. Points within triangles are as explained in legend of figure 2.

Fig. 4. Comparison of curve of daily per cent weight increase and curve of transfer rate of  $H_2O$  per unit weight of fetus during the gestation period. Points in triangle are as explained for figure 2.

unit weight of the 66-day placenta as across the same weight of the 28-day placenta.

*Variation of transfer per unit weight fetus with respect to fetal weight and relative growth rate.* The method of calculation of the relative growth rate of the guinea pig fetus has been reported (1). The transfer rate of  $H_2O$  per unit weight of fetus can be readily obtained from figure 2. Figure 4 shows the variation in these two quantities with increase of fetal weight. The experimental determinations of the transfer rate per unit weight of fetus show considerable scattering and so fix the position of the curve in a less precise way than was generally the case with sodium (1; 5 to 9).

The two curves during that period of gestation which has been investigated are similar, the ratios of their ordinates having the following values: at a fetal weight of 1 gram, 2; at 10 mgm., 3; at 40 mgm., 3; at 70 grams, 3 and at 90 grams, 3. It appears consequently that the hypothesis advanced as a result of

our experience with sodium holds also for water, i.e., that the fundamental principle underlying placental transfer is that the rate of supply of a substance to a unit weight of fetus shall parallel the rate at which that unit weight of fetus is reproducing itself.

*Fetal need for H<sub>2</sub>O relative to supply across placenta.* The ratio of the quantity of a substance supplied to the fetus to the amount of that substance retained by the fetus in its growth has been called the safety factor for that substance (1). The safety factor for water is readily calculated from the data of table 2. The quantity of water reaching the fetus across the placenta per hour is calculated from the relative content of DHO of the fetus and the maternal blood as explained above. The quantity of water retained by the fetus in an hour is equal to the total water content of the fetus multiplied by the daily per cent weight increase and divided by 24 times 100. The safety factor for water, as can be seen in table 2, varies from about 150 for a fetus of 1 gram to 500 for a fetus of 100 grams, i.e., the fetus, depending on its size, receives water across the placenta

TABLE 2  
*Safety factor for water at various fetal weights*

FETAL WEIGHT	DAILY PER CENT WEIGHT INCREASE	TOTAL H <sub>2</sub> O CONTENT OF FETUS	TOTAL H <sub>2</sub> O TRANSFERRED TO FETUS PER HOUR	TOTAL H <sub>2</sub> O RETAINED IN HOURLY GROWTH OF FETUS	SAFETY FACTOR
<i>grams</i>		<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	
100	10	72	160	0.30	533
30	10	26	54	0.11	490
3	22	2.7	6	0.025	240
1	33	0.92	2	0.013	154

in remarkably high quantities amounting to from 150 to 500 times that retained in growth.

DISCUSSION. The increase in transfer rate across a unit weight of placenta from the twenty-eighth day of gestation to term, or shortly before, is of the same order of magnitude both for H<sub>2</sub>O and Na (1). The overall increase for Na during this period was found to be about 10 times; that for water, about 9 times. Intermediate points between these two stages of pregnancy on the H<sub>2</sub>O-transfer and Na-transfer curves are not related as exactly. This is due to the absence in the series of observations with DHO of the apparent minimum in the curve found with Na<sup>24</sup> at a gestation age of 50 days. The morphological and physiological changes which can be correlated with the increase of unit placental transfer rates as pregnancy proceeds have been given (1).

The results of the experiments with Na<sup>24</sup> led to the hypothesis that the fundamental principle underlying placental transfer is that the rate of transfer of a substance to a unit weight of fetus shall parallel the relative growth rate of the fetus. This hypothesis was satisfied by the observations on guinea pig, rat, rabbit, cat, goat and sow (1; 5 to 9) during the period of the experiments which was approximately the last half of gestation. The results on water transfer to

the fetus of the guinea pig also fit the hypothesis and so give to it considerable additional strength.

The rate at which  $H_2O$  is supplied to the fetus across the placenta is considerably greater than that for  $Na$ . This is reflected in differences in the times necessary for the fetus to come to equilibrium with DHO and  $Na^{24}$  in the maternal blood. Although DHO is distributed throughout the fluids of the fetus whereas  $Na^{24}$  is limited to the extracellular fluid, DHO injected intravenously into the mother comes to within 80 per cent of equilibrium with the fetus in about half an hour in contrast to  $Na^{24}$  which requires four hours for the same degree of equilibration. The greater rate of supply of  $H_2O$  to the fetus is also reflected in its higher safety factor which varies from 150 in early stages to 500 near term as compared to values of 40 and 65 for the safety factor for  $Na$  at equivalent stages of gestation.

The actual amount of  $H_2O$  transferred to the fetus per hour is given in figure 2 above; the amount of  $Na$  supplied has previously been given (1). If the placenta were equally permeable to  $H_2O$  and to  $Na$  and if it performed no secretory work in their transfer, the concentration of  $Na$  in the fluid delivered to the fetus would be the same as that in the maternal plasma, i.e., 330 mgm. of  $Na$  per 100 cc. of  $H_2O$ . The concentration of  $Na$  delivered to the fetus is, however, far below this level. The 100 gram fetus, for example, receives about 170 cc. of  $H_2O$  per hour and only 26 mgm. of  $Na$ . The anticipated quantity of  $Na$  on the basis of equal placental permeability to  $H_2O$  and  $Na$  and no secretory work is 560 mgm.

Though at first glance this may be taken as evidence for placental secretory activity, it can also be completely explained by assuming a greater placental permeability to  $H_2O$  than to  $Na$ . In order to clarify this latter view we have performed the following experiment with a collodion membrane. The membrane had a surface area of 16 sq. cm. and was in the form of a sac. It was immersed in and filled with a one per cent solution of  $NaCl$ . To measure the relative rate of movement of  $H_2O$  and  $Na$  across the membrane under this condition of equilibrium, we have added a small quantity of  $Na^{24}Cl$  in  $D_2O$  to the inside of the membrane. Under conditions of adequate stirring, samples were then taken from the inner and outer liquids and analyzed for DHO and  $Na^{24}$ .

The difference in rate of movement of  $H_2O$  and  $Na$  across the membrane at equilibrium may be judged from the following observations made 15 minutes after the start of the experiment. One cubic centimeter of fluid from inside the membrane contained 0.869 per cent DHO and had a level of radioactivity of 16,000 beta-rays per second. One cubic centimeter from outside (total volume 5 cc.) the membrane contained 0.086 per cent DHO and had a radioactivity of 203 beta-rays per second. From these values it can be calculated as explained above that 125 mgm.  $H_2O$  and 0.16 mgm. of  $Na$  moved across a square centimeter of membrane per hour. If the membrane were equally permeable to  $H_2O$  and  $Na$ , 1.25 mgm. of  $Na$  would move with 125 mgm. of  $H_2O$  as this is their ratio of concentration in the solution. It has been demonstrated, consequently, that at equilibrium the relative rate of movement of  $H_2O$  and  $Na$  back and forth across the membrane is not equal to their concentration ratio. The concentra-

tion ratio of  $\text{H}_2\text{O}$  to Na in the solution was 99 to 1; the ratio of  $\text{H}_2\text{O}$  to Na which moved through a unit area of membrane in a unit time was 780 to 1.

To make this experiment comparable to the situation in the placenta, it is necessary to consider the consequences of applying pressure of the order of magnitude of capillary pressure to the fluid within the sac. Under this condition, the volume of fluid outside the sac will increase at the expense of that within. As has been shown (10), the concentration of  $\text{H}_2\text{O}$  and Na and so of DHO and  $\text{Na}^{24}$  in the fluid pressed across the membrane in this way will be indistinguishable from that in the mother liquor. The total amount of DHO and  $\text{Na}^{24}$  which would move into the outer liquid in this case would, however, not be in the ratio of their concentrations within the membrane. This is so because superimposed upon that which moves as a result of the pressure is transfer of the sort which occurs at equilibrium.

This last statement holds also for transfer of  $\text{H}_2\text{O}$  and Na from the maternal to the fetal circulation across the placenta. There may in addition be secretory activity by the placenta through which  $\text{H}_2\text{O}$  is separated from Na. It is impossible to make any conclusion as to this latter possibility from the present data.

#### SUMMARY

1. Changes in rate of placental transfer of  $\text{H}_2\text{O}$  have been measured from the 28th day of pregnancy until term, using DHO as the tracer substance. The transfer rate of  $\text{H}_2\text{O}$  per unit weight of placenta increases about 9 times during this period.

2. The results with DHO satisfy the hypothesis, previously advanced for Na, that the fundamental principle underlying placental transfer is that the rate of transfer to a unit weight of fetus shall parallel the relative growth rate of the fetus.

3. The fetus in the early stages studied here receives across the placenta about 150 times as much  $\text{H}_2\text{O}$  and in the later stages almost 500 times as much  $\text{H}_2\text{O}$  as is incorporated in the growing tissues.

We are grateful to Dr. David Rittenberg for generous advice about the apparatus.

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# THE TRANSFER OF WATER AND SODIUM TO THE AMNIOTIC FLUID OF THE GUINEA PIG

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The results of measurement of the rate of transfer of sodium and of water across the placenta of the guinea pig to the fetus have been reported (1, 2). The present series of experiments was undertaken to determine the rate at which water and sodium are transferred from the maternal circulation to the amniotic fluid. Radioactive sodium in the form of chloride ( $\text{Na}^{24}\text{Cl}$ ) and heavy water ( $\text{D}_2\text{O}$ ) were used as the tracer materials.

**METHOD.** Eight pregnant guinea pigs were used to study the transfer of water to the amniotic fluid. Heavy water (95 per cent  $\text{D}_2\text{O}$ ) was injected intravenously in such amounts as usually give a final concentration in the blood of about 0.6 per cent. From preliminary experiments it was found that the exchange of heavy water between the maternal circulation and the amniotic fluid was linear up to 30 minutes. Therefore, in the transfer experiments, the abdomen was opened under light ether anesthesia, the uterus and chorion incised, and the amniotic sac exposed 10 to 30 minutes after the heavy water had been injected into the mother. As much amniotic fluid as possible was withdrawn by means of a hypodermic needle and syringe, care being taken to avoid contamination of the fluid by blood. A sample of maternal blood was secured by cardiac puncture. The water of the amniotic fluid and of the maternal blood was obtained by vacuum distillation to dryness at room temperature. The purification of the water so obtained, and the method of determining the concentration of heavy water by the falling drop method were as described by Keston, Rittenberg and Schoenheimer (3).

The rate of transfer of radioactive sodium to the amniotic fluid was measured in a second series of eight pregnant guinea pigs. Three to five microcuries of radioactive sodium, depending on the weight of the mother, were injected intravenously. From preliminary experiments, three hours was found to be the optimum interval at which to terminate the experiment. The amniotic fluid and maternal blood were secured in the same way as in the heavy water experiments. The radioactivity present in the samples was measured in a pressure ionization chamber-string electrometer apparatus in a manner previously reported (1).

In both series of experiments the observations were carried out on animals at progressive periods of gestation from about the end of the first third of pregnancy to term.

**RESULTS.** The method and rationale for calculating the rate of transfer of water to the fetus have been given (2). Similarly the amount of water trans-



ferred to the amniotic fluid in a known interval of time may be calculated from the equation:

$$H_2O_{AmF} = DHO_{AmF} \times Vol_{AmF} \div DHO_{MB}$$

where  $H_2O_{AmF}$  represents the total water transferred to the amniotic fluid;  $DHO_{AmF}$ , the observed concentration of heavy water in the water of the amniotic fluid;  $DHO_{MB}$ , the average concentration, during the period of the experiment, of heavy water in the water of the maternal blood. The average concentration of heavy water in the water of the maternal blood is used in the calculation because of the rapidly changing concentration of DHO in the blood during the

TABLE 1

*Rate of transfer of water to amniotic fluid from maternal circulation*

Litter mates are grouped together. "Delivery time of amniotic fluid" refers to minutes after injection of  $D_2O$  into mother.

FETAL WEIGHT	DELIVERY TIME OF AmF	OBSERVED CONCENTRATION DHO IN BLOOD	AVERAGE CONCENTRATION DHO IN BLOOD	CONCENTRATION DHO IN AmF	VOLUME AmF	TOTAL WATER TRANSFERRED TO AmF/HR.	RATE OF TURNOVER OF AmF/HR.
<i>grams</i>	<i>minutes</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>cc.</i>	<i>cc.</i>	
0.8	10.5	0.657	1.11	0.171	1	0.9	0.9
7.5	22.4	0.062	0.076	0.031	5	5	1.0
10.8	30.0	0.747	0.928	0.480	6	6	1.0
11.0	30.5			0.535	6	7	1.2
11.0	30.7			0.565	6	7	1.2
12.4	23.2	0.324	0.426	0.100	6	4	0.7
13.8	17.2	0.570	0.810	0.400	7	11	1.6
20.0	28.0	0.224	0.283	0.202	8	12	1.5
26.0	12.8	0.670	1.04	0.281	9	11	1.2
105.0	11.0	0.618	1.02	0.321	16	28	1.7

early part of the experiment. The method for obtaining this value has been reported (2). The volume of the amniotic fluid ( $Vol_{AmF}$ ) at different periods of gestation has been taken from Ibsen (4).

Table 1 gives the results of the experiments on the rate of transfer of water to the amniotic fluid. As can be seen, in the earliest stage of gestation observed (fetal weight 0.8 gram, estimated gestation age 30 days), 0.9 cc. of water was transferred to the amniotic fluid per hour; at term (fetal weight 105 grams), 28.5 cc. of water was transferred to the amniotic fluid per hour. Hence there is an increase of about 30 times in the rate of transfer of water to the amniotic fluid from the earliest to the latest stage of pregnancy studied. Although the

rate of transfer of water at later stages is much greater than at earlier stages, the overall result is much the same inasmuch as a volume of water approximately equal to the total volume of the amniotic fluid flows into and out of the amniotic sac about once an hour in the earlier stages (up to a fetal weight of 13 grams) and about once every 40 minutes in the later stages.

The method of calculating the amount of sodium transferred to the fetus from the transfer rate of  $\text{Na}^{24}$  to the fetus has been reported (1). In like manner the

TABLE 2

*Rate of transfer of sodium to amniotic fluid from maternal circulation*

Litter mates are grouped together. "Delivery time of amniotic fluid" refers to hours after injection of  $\text{Na}^{24}$  into mother. The radioactivities have been measured in samples larger than 1 cc. but are presented in this unit for convenience.

FETAL WEIGHT	DELIVERY TIME OF AmF	$\text{Na}^{24}$ /cc. MATERNAL PLASMA	$\text{Na}^{24}$ /cc. AmF	Na TRANSFERRED PER cc. AmF/HR.	VOLUME AmF	TOTAL TRANSFER Na TO AmF/HR.	RATE OF TURNOVER OF Na OF AmF/HR.
grams	hours	beta-rays/sec.	beta-rays/sec.	mgm.	cc.	mgm.	
3.3	3.17	986	24	0.025	3	0.08	0.011
3.5			20	0.021	3	0.06	0.009
7.1	3.08	1230	30	0.026	5	0.13	0.011
7.0			33	0.028	5	0.14	0.012
7.0			25	0.021	5	0.11	0.009
9.3	3.17	1105	23	0.022	6	0.13	0.009
31.5	3.30	1235	61	0.050	9	0.45	0.021
31.5			73	0.060	9	0.54	0.025
31.5			69	0.057	9	0.51	0.024
31.0	6.25	323	18	0.030	9	0.27	0.013
30.0			28	0.047	9	0.43	0.020
32.0			22	0.037	9	0.33	0.015
34.5	3.88	1900	125	0.057	10	0.57	0.024
42.0	3.00	58	1.7	0.033	10	0.33	0.014
69.4	3.33	866	136	0.160	12	1.9	0.067
70.0			87	0.100	12	1.2	0.042

amount of sodium transferred to the amniotic fluid for the time period of the experiment can be calculated from the equation:

$$\text{Na}_{\text{AmF}} = \text{Na}_{\text{AmF}}^{24} \times \text{Conc. Na}_{\text{MP}} \times \text{Vol}_{\text{AmF}} \div \text{Na}_{\text{MP}}^{24}$$

where  $\text{Na}_{\text{AmF}}^{24}$  and  $\text{Na}_{\text{MP}}^{24}$  refer to the radioactivities present in 1 cc. of amniotic fluid and maternal plasma.

We have assumed the concentration of sodium in the maternal plasma to be 330 mgm. per 100 cc. No correction need be made for non-diffusible sodium in

the plasma because in this regard both Na and  $\text{Na}^{24}$  are affected alike. The results on the rate of transfer of sodium to the amniotic fluid are given in table 2. The calculations of the rate at which the sodium of the amniotic fluid is replaced by sodium of the maternal plasma have been made on the assumption that there are 2.4 mgm. Na per cc. of amniotic fluid. This value was obtained from Needham (5). There is a marked increase of between 20 and 30 times in the total amount of sodium transferred per hour to the amniotic fluid from the early stages to term. As can be seen from the values in the final column of table 2, the rate of turnover of the sodium at all stages of pregnancy is low; only about one-fiftieth of the sodium is replaced on the average per hour.

DISCUSSION. The rate at which water is transferred to the amniotic fluid is considerably greater than that for sodium. This is reflected in the difference in rate of turnover of water and sodium in the amniotic fluid. Whereas the amount of water which flows into and out of the amniotic sac in an hour is approximately equal to the volume of the amniotic fluid at all stages of gestation, only about one-fiftieth of the sodium is replaced in the same time interval. This means that water is replaced on the average 50 times more rapidly than sodium. When a comparison of the rates of transfer of water and sodium across the placenta of the pregnant guinea pig was made, a similar difference was noted (2). Evidence was given which could explain completely the observed discrepancy on the basis of greater permeability of the placental membrane to water than to sodium. The same explanation, applied to whatever membranes are involved, may hold for the difference of transfer rates of water and sodium from the maternal circulation to the amniotic fluid.

The main source of the amniotic fluid has been ascribed by some investigators to fetal urine. The present experiments shed no light as to the principal site at which the exchange of water and sodium between the maternal blood and amniotic fluid takes place. Since in several of the earlier stages, a volume of water equal to that of the fetus (see table 1) flows in and out of the amniotic sac in an hour, it would seem questionable that the fetal urine is the principal source of the amniotic fluid. The astonishingly rapid rate of replacement of the water of the amniotic fluid is also at variance with our previous concept that the amniotic fluid is relatively stagnant.

#### SUMMARY AND CONCLUSIONS

The rate of passage of water and sodium from the maternal circulation to the amniotic fluid has been measured from the end of the first third of pregnancy to term. Radioactive sodium as the chloride ( $\text{Na}^{24}\text{Cl}$ ) and heavy water ( $\text{DHO}$ ) were used as the tracer substances.

The rate at which water is delivered to the amniotic fluid is such that a volume of water equal to the volume of the amniotic fluid is exchanged on the average about once an hour at all periods of gestation examined.

The rate of transfer of sodium to the amniotic fluid at various stages of gestation is on the average about 50 times less rapid than the water transfer.

We wish to express our gratitude to Dr. Dean B. Cowie of the National Cancer Institute and Dr. B. R. Curtis of the Jefferson Physics Laboratory, Harvard University, for preparing the radioactive sodium with the Carnegie electrostatic generator and the Harvard cyclotron respectively.

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# STUDIES ON THE RELATION OF THE LIVER FUNCTION, PULSE RATE AND TEMPERATURE OF HYPERTHYROID DOGS TO VITAMIN B<sub>1</sub> AND YEAST<sup>1,2</sup>

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Evidence has accumulated to show the increasing importance of the B vitamins in both experimental and clinical hyperthyroidism. Increased requirements of undifferentiated vitamin B during hyperthyroidism was demonstrated by Himwich, Goldfarb and Cowgill (16), and Sure and co-workers (26, 27) have shown that crystalline vitamin B<sub>1</sub> will partially protect thyroid fed rats from a loss of weight. Drill (9) and Drill and Sherwood (13) have reported that thyroid fed rats which had lost weight will regain their normal weight, when *both* vitamin B<sub>1</sub> and yeast are administered, even though thyroid feeding is continued. It was also found that a normal estrus cycle could be restored in thyroid fed rats with the administration of the B vitamins. Peters and Rossiter (22) confirmed the report of Drill (10) that thyroid fed rats show a decrease of vitamin B<sub>1</sub> in their tissues. The administration of yeast to thyroid fed rats will also prevent the fall of liver glycogen that usually occurs (8). It has recently been reported that the calorie intake and the maintenance of weight of hyperthyroid dogs depends on the intake of the B vitamins (11). Means et al. (20), Frazier and Ravdin (15), and Scrutinio (25) have used crystalline vitamin B<sub>1</sub> and yeast in cases of human hyperthyroidism and have reported beneficial results.

The experimental work with the B vitamins has thus far been concerned only with some of the symptoms of hyperthyroidism, such as weight change, food intake, estrus cycle, liver glycogen, and the tissue storage of vitamin B<sub>1</sub>. The liver damage, pulse rate, and body temperature of hyperthyroid animals have not as yet been studied in relation to the B vitamins, and will be reported here.

It has been shown that the liver is altered both structurally (14) and functionally (5) during the hyperthyroid state. Only the functional changes will be considered here. Youmans and Warefeld (30), Maddock et al. (19), and Bartels (3) have clearly demonstrated the presence of an abnormal liver function in a majority of hyperthyroid patients (cf. 14). The above authors also noted that the liver damage increased with the severity of the disease. The work of Sanger and Hun (24) indicates that the hyperthyroid patient fails to store glucose as liver glycogen. Kugelman (18) studied the effect of levulose on the blood sugar curve of normal and hyperthyroid patients and concluded that the liver of the

<sup>1</sup> The authors wish to thank Eli Lilly and Company for a grant in support of part of these experiments.

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hyperthyroid individual failed to store glycogen or even to convert the levulose into glucose, a process which normally takes place in the liver. Experimental work has demonstrated that feeding thyroid gland to animals greatly reduces the amount of glycogen in the liver. This was first shown by Cramer and Kraus (7) and by Parhon (21) and has been confirmed on nearly every type of experimental animal (cf. 14). It has since been found that the administration of the B vitamins, as yeast, will prevent the loss of liver glycogen that usually occurs in hyperthyroid rats (1, 2, 8).

This evidence shows the increasing importance of the liver and of the B vitamins in both clinical and experimental hyperthyroidism. It was thus decided to investigate possible relationships between the liver function of hyperthyroid dogs and the B vitamins. This was done with the hope of obtaining additional knowledge of the cause of the abnormal liver function in patients with Graves' disease. Studies were also made on the effect of the B vitamins on the pulse rate and temperature of the hyperthyroid dogs. A preliminary report on the liver function studies has been published (12).

**METHODS.** Male dogs weighing between 9 and 17 kgm. were used as experimental animals. A sex difference in response to thyroid feeding in rats has been reported (9), and although no such difference has been reported in dogs, for the sake of conformity and interpretation of results animals of only one sex were used. Lilly's desiccated thyroid gland,<sup>4</sup> containing 0.21 per cent of iodine, was used, the dosages employed being listed in table 1. Two weeks before the thyroid feeding was started the dogs were placed on a modified form (12) of Cowgill's casein diet no. III (6), which is free of the B vitamins. This diet was fed for a three hour period each day, the amount eaten being measured. Each dog also received a daily supplement of a dried baker's yeast.<sup>5</sup> The yeast contained 23 International Units of vitamin B<sub>1</sub> per gram,<sup>5</sup> and was fed in a dosage of 4.4 I.U. of vitamin B<sub>1</sub> per kgm. of body weight.

All the animals were fed a synthetic diet with the minimal amount of yeast that would still afford normal maintenance in the untreated dog. Thus, the increased requirements for the B vitamins caused by thyroid feeding would result in a progressive chronic deficiency of the B vitamins. At various time intervals during thyroid feeding the yeast was removed from the diet of the dogs and the effect on the liver function, pulse rate, and rectal temperature noted. Crystalline vitamin B<sub>1</sub> was then injected in large doses, and later a yeast concentrate was added to the diet, and changes in the liver function, pulse rate and temperature were studied.

Liver functions were determined by the bromsulphalein method of Rosenthal and White (23), except that 5 mgm. of bromsulphalein per kilogram of body

<sup>4</sup> The authors are indebted to Dr. C. N. Frey of The Fleischmann Laboratories for supplying the analysed yeast and yeast concentrate, to Dr. R. T. Major of Merck and Company for supplying the crystalline vitamin B<sub>1</sub>, and to Dr. H. W. Rhodehamel of Eli Lilly and Company for the desiccated thyroid gland.

<sup>5</sup> The dried baker's yeast also contained 60 gamma of riboflavin, 85 gamma of vitamin B<sub>6</sub>, and 120-150 gamma of calcium pantothenate per gram.

weight, instead of 2 mgm., were injected intravenously into trained dogs, and a single blood sample was withdrawn under oil after a one-half hour interval. In the standards 4 mgm. of bromsulphalein per 100 cc. of dilute NaOH was used to represent 100 per cent. Readings above 100 per cent retention of dye are obtainable during liver damage, since the colorimeter standards, originally developed for the dosage of 2 mgm. of dye per kilogram of body weight, were retained with the 5 mgm. dose. The greater difficulty in comparison of the solutions made the lower concentration of standard preferable.

RESULTS. The normal dogs in table 1 gained weight and were maintained in perfect health during the experiment, showing that the diet and yeast supplement were adequate. The liver function tests in the normal dogs and in the experimental animals before thyroid feeding generally showed between 2 to 8 per cent retention of dye. Control values above 12 per cent were not obtained in the dogs. Therefore we have regarded a dye retention above 15 per cent as definitely abnormal.

TABLE 1

DOG NO.	WEIGHT	THYROID FED PER KGM. OF BODY WEIGHT	YEAST FED PER DAY	DAY OF EXP. YEAST REMOVED FROM DIET
	<i>kgm.</i>	<i>grams</i>	<i>grams</i>	
1	10.72	None	2.0	Not removed
2	16.22	None	3.1	Not removed
3	11.97	0.4	2.2	59
4	10.12	0.4	1.9	80
5	14.29	0.4	2.7	57
6	13.84	0.4	2.6	29
7	13.61	0.6	2.6	44
8	8.90	0.6	1.7	41
9	12.90	0.6	2.4	50

1. *Liver function.* *Effect of thyroid feeding and removal of yeast from the diet*  
At least three control liver function determinations were made on the experimental animals before thyroid feeding was started. Figure 1 shows the comparative effects of thyroid feeding and the removal of the yeast from the diet on liver function. One dog (no. 9) showed no abnormal dye retention while the thyroid was being fed or after the yeast was removed from the diet. This dog had received thyroid gland for a total of 69 days. Dogs 4, 8, and 3 gave abnormal liver functions on the 34th, 41st and 59th day of thyroid feeding, respectively, before the yeast was removed from the diet. The removal of the yeast from the diet of dog 8 did not further increase the dye retention (fig. 1), whereas the removal of the yeast from the diet of dog 3 did increase the dye retention.

Dog 5 maintained a normal liver function until the 57th day of thyroid feeding. The yeast was then removed from the diet on the 57th day of the experiment and the following day the dye retention was 20 per cent, which slowly increased as the thyroid feeding was continued. The removal of the yeast from the diet of dog

6 also produced an abnormal dye retention which progressively increased. In dog 7 one-half of the yeast was removed on the 42nd day of thyroid feeding, and 24 hours later the liver function test showed 25 per cent retention of dye. On the 44th day, all of the yeast was removed and the following day 150 per cent retention of dye was obtained. This value then dropped and remained between 50 to 80 per cent retention of dye. Thus, the removal of the yeast from the diet

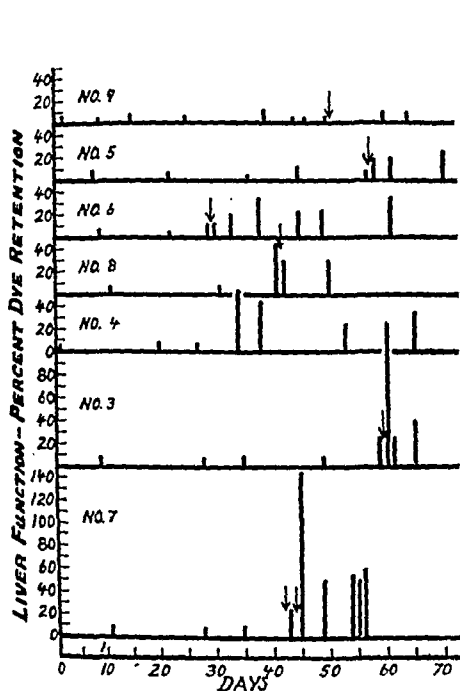


Fig. 1

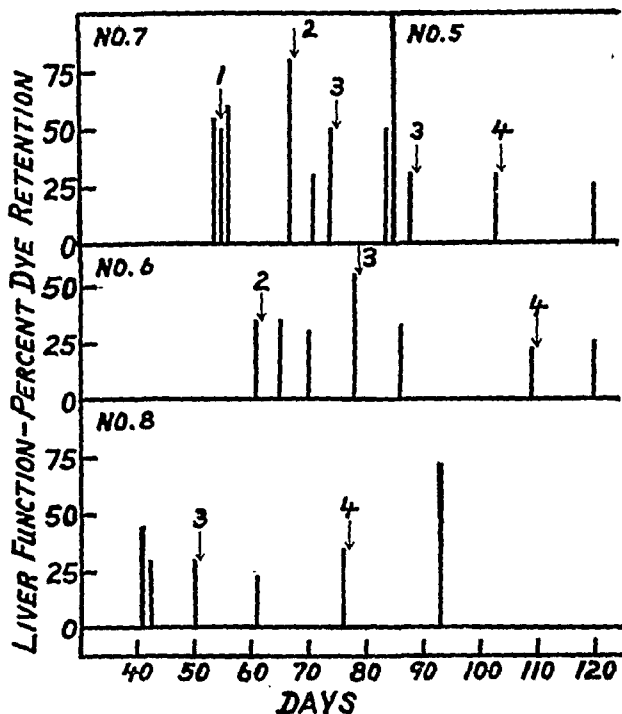


Fig. 2

Fig. 1. The effect of yeast removal from the diet on the liver function of hyperthyroid dogs. The arrow indicates the removal of the yeast from the diet. In dog 7 the first arrow indicates the removal from the diet of one-half of the yeast and the second arrow of all of the yeast.

Fig. 2. The effect of yeast supplements, vitamin B<sub>1</sub> injections, and yeast concentrate on the abnormal liver function produced in hyperthyroid dogs. 1. The effect of a single injection of 2 mgm. of vitamin B<sub>1</sub>. 2. The effect of a single injection of 2 mgm. of vitamin B<sub>1</sub> and the replacement of the original amount of yeast in the diet. 3. The addition of 10 grams of yeast concentrate per day to the diet. 4. The addition of 10 grams of yeast concentrate to the diet each day plus the daily injection of 1 mgm. of vitamin B<sub>1</sub>.

seems to have a causal relationship to the production of an abnormal liver function in thyroid fed dogs.

*Effect of vitamin B<sub>1</sub> and yeast on liver function.* After the abnormal liver functions had developed in the thyroid fed dogs, they were treated by injections of vitamin B<sub>1</sub>, and by the addition of a yeast concentrate to their diet. The materials administered and the results obtained are illustrated by four dogs in figure 2. All of the dogs had previously had the yeast removed from their diet. The single subcutaneous injection of 2 mgm. of vitamin B<sub>1</sub> gave no change in the liver



function. When, in addition to the single subcutaneous injection of vitamin B<sub>1</sub>, the original amount of yeast was restored to the diet, there was no correcting influence on the abnormal liver function. Even when 10 grams of a yeast concentrate per day,<sup>6</sup> or 10 grams of a yeast concentrate each day plus 1 mgm. of vitamin B<sub>1</sub> subcutaneously per day were given, there was no reduction in the percentage of dye retention (fig. 2). Thus the abnormal liver function of the thyroid fed dogs, which had been "precipitated" by removing the yeast from the diet, was not improved by the large amount of yeast concentrate and the vitamin B<sub>1</sub> injections that were administered each day.

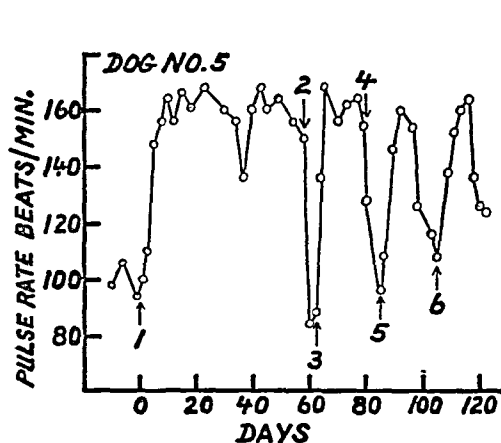


Fig. 3

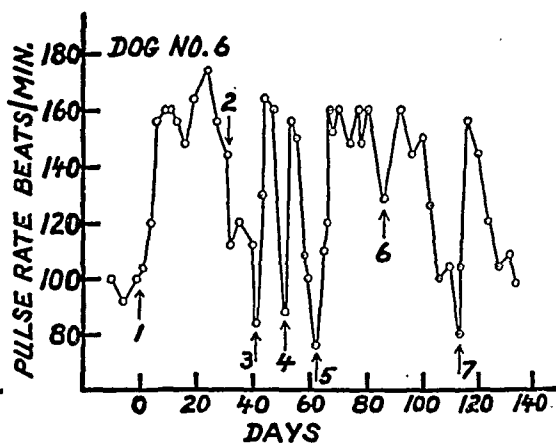


Fig. 4

Fig. 3. The effect of vitamin B<sub>1</sub> and yeast on the pulse rate of a hyperthyroid dog. 1. Thyroid feeding started. 2. Yeast removed from the diet. 3. Two milligrams of vitamin B<sub>1</sub> injected and yeast replaced in the diet. 4. Yeast removed from the diet. 5. Ten grams of yeast concentrate fed each day. 6. Ten grams of yeast concentrate fed each day plus a daily injection of 1 mgm. of vitamin B<sub>1</sub>.

Fig. 4. The effect of vitamin B<sub>1</sub> and yeast on the pulse rate of a hyperthyroid dog. 1. Thyroid feeding started. 2. Yeast removed from the diet. 3. Effect of a single injection of 2 mgm. of vitamin B<sub>1</sub>. 4. Effect of a single injection of 2 mgm. of vitamin B<sub>1</sub> with the replacement of the yeast in the diet each day. 5. The injection of 1 mgm. of vitamin B<sub>1</sub> each day with the yeast still in the diet. 6. Dog fed 10 grams of yeast concentrate per day. 7. Dog fed 10 grams of yeast concentrate per day plus the injection of 1 mgm. of vitamin B<sub>1</sub> each day.

2. *Pulse rate. Effect of thyroid feeding and removal of yeast from the diet.* The two control dogs and the seven experimental dogs all had normal pulse rates of 80-105 beats per minute. When thyroid feeding was started six of the seven dogs gave a slow but steady increase in pulse rate from the normal, reaching, in 6 to 8 days, a rate of 150 to 160 beats per minute (figs. 3, 4, 6). The seventh dog showed some tachycardia, but the pulse rate was irregular and varied from 100 to 160 beats per minute during the first 54 days of thyroid feeding. In the six dogs with tachycardia, the pulse rate generally varied from 150 to 160 beats per minute, sometimes going as low as 140 beats per minute. When the yeast was re-

<sup>6</sup> The yeast concentrate contained 200 U.S.P. units of vitamin B<sub>1</sub>, 230 gamma of riboflavin, 200 gamma of vitamin B<sub>6</sub>, and 1500-2000 gamma of calcium pantothenate per gram.

moved from the diet, all of the hyperthyroid dogs showed a marked decrease in pulse rate, dropping to as low as 74 to 80 beats per minute, while still receiving thyroid gland (figs. 3, 4, 5, and table 1).

*Effect of vitamin B<sub>1</sub> and yeast on pulse rate.* After the yeast had been removed from the diet of the hyperthyroid dogs, and the pulse rate had fallen, each dog received an injection of 2 mgm. of vitamin B<sub>1</sub>. Within 48 hours after the injection, the pulse rate again rose to between 140 and 160 beats per minute (figs. 3, 4). As soon as the injected vitamin B<sub>1</sub> was metabolized and/or excreted the pulse rate again fell to low levels (figs. 3, 4). It could again be raised to the hyperthyroid level by another injection of vitamin B<sub>1</sub>.

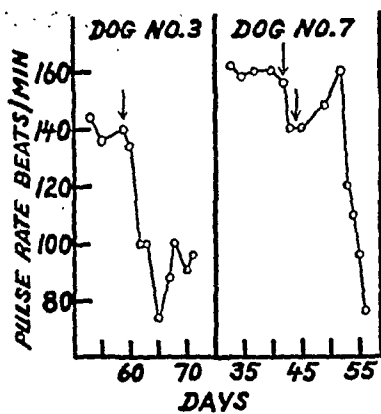


Fig. 5

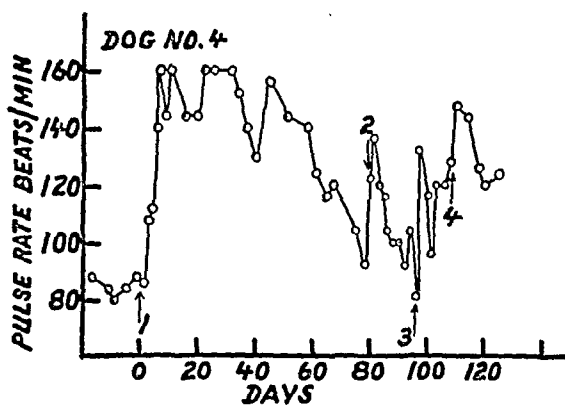


Fig. 6

Fig. 5. The effect of removal of the yeast from the diet on the pulse rate of hyperthyroid dogs. Arrow indicates removal of the yeast from the diet. In dog 3 the first arrow indicates the removal of one-half of the dietary yeast and the second arrow the removal of all of the yeast.

Fig. 6. Effect of vitamin B<sub>1</sub> and yeast on the pulse rate of a hyperthyroid dog. 1. Thyroid feeding started. 2. Yeast removed from the diet. Note the decline in pulse rate before the yeast was removed. 3. One milligram of vitamin B<sub>1</sub> injected and yeast replaced in the diet. 4. One milligram of vitamin B<sub>1</sub> injected each day plus a supplement of 10 grams of a yeast concentrate per day. The pulse rate is still above normal but no marked tachycardia is present.

The dogs were then injected with vitamin B<sub>1</sub> and the initial amount of yeast was replaced in the diet. The pulse rate again rose only to fall when the administered vitamin B<sub>1</sub> was metabolized or excreted. Thus the original amount of yeast was not sufficient to maintain the characteristic pulse in the hyperthyroid condition. When 10 grams of a yeast concentrate<sup>6</sup> was substituted for the original amount of yeast, a similar result was obtained, although the fall in pulse rate was much slower (figs. 3, 4, 6). Finally, when a daily injection of 1 mgm. of vitamin B<sub>1</sub> was given in addition to the yeast concentrate, a marked tachycardia was again observed. However, when the thyroid feeding was continued for 90-100 days, despite the supplements of vitamin B<sub>1</sub> and yeast, the pulse rate again began to fall.

It has since been observed that dogs fed thyroid gland for 120 days, and re-

ceiving yeast concentrate and vitamin B<sub>1</sub> injections throughout the experiment, will maintain a tachycardia for only 60 to 90 days, after which time the pulse rate drops to an average of 120 beats per minute. The drop in pulse rate seems to be associated with a hypertrophy of the heart.<sup>7</sup> It is evident, therefore, that during the first 60 days of thyroid feeding the pulse rate of the hyperthyroid dogs is dependent on an adequate supply of vitamin B<sub>1</sub>. The pulse rate falls when the yeast is removed from the diet during the first 60 days and rises when vitamin B<sub>1</sub> is injected (figs. 3, 4). Thus the tachycardia is not a specific effect of thyroid feeding per se, but depends also on an adequate supply of vitamin B<sub>1</sub>.

3. *Rectal temperature.* The rectal temperature of the control dogs and the experimental animals, before thyroid gland was fed, averaged 38.6°C. (table 2). When thyroid feeding was started, there was an average rise in rectal temperature of 0.5°C during the first 20 days. The rectal temperature still increased slowly

TABLE 2  
*Average rectal temperature of hyperthyroid dogs °C.*

DOG NO.	THYROID FED PER KG.M. OF BODY WEIGHT	BEFORE THYROID FEEDING	DAYS OF THYROID FEEDING BEFORE YEAST WAS REMOVED			YEAST REMOVED	VITAMIN B <sub>1</sub> INJECTED
			20	30	40		
	<i>grams</i>						
1	None		38.0-38.5 throughout the experiment				
2	None		38.2-39.0 throughout the experiment				
3	0.4	38.6	39.0	39.7	40.0	39-39.5	39.5
4	0.4	38.4	38.8	38.9	38.9	38.6	38.8
5	0.4	38.6	39.0	39.1	39.1	38.8	38.7
6	0.4	39.0	39.7	39.8		39.0	39.5
7	0.6	38.5	38.8	39.0	39.9	38.5	39.0
8	0.6	38.6	39.4	39.6	39.8		
9	0.6	38.3	38.9	38.9	39.0	38.7	
Average.....		38.6	39.1	39.3	39.45	38.8	39.1

until about the fortieth day. When the yeast was removed from the diet of the hyperthyroid dogs, a drop in rectal temperature of from 0.3 to 1.5°C. was observed, the average fall in temperature being 0.6°C. Coincident with this drop in temperature there was a drop in food intake (11).

The injection of vitamin B<sub>1</sub> increased the rectal temperature 0.2 to 0.5°C, the average being 0.3°C (table 2). The rise in rectal temperature paralleled the increase in food consumption which follows the injection of vitamin B<sub>1</sub>. This seems to indicate that the change in rectal temperature is a reflection of the changes in food intake, which in this case depends on the amount of vitamin B<sub>1</sub> that is supplied (11).

DISCUSSION. The dogs used in this experiment were fed a synthetic diet containing a minimal but normal supplement of analysed yeast, so that each dog received 2 International Units of vitamin B<sub>1</sub> per pound of body weight. This

<sup>7</sup> V. A. Drill, C. B. Shaffer and R. Overman, to be published.

amount of yeast maintained a normal food intake and allowed a slight gain in weight in the control dogs. However, since the feeding of thyroid gland increases the requirements for vitamin B<sub>1</sub> (11, 13, 16, 27), the hyperthyroid dog fed this small amount of yeast will soon have a relative deficiency of the B vitamins. This experimental procedure was chosen because, in view of the reports of the low intake of the B vitamin in the American diet, it was unlikely that the average untreated patient with Graves' disease was receiving a sufficient supply of the B vitamins.

Out of seven thyroid fed dogs, one failed to develop any abnormal liver function during the experiment, even when the yeast was removed from the diet. Three of the thyroid fed animals developed abnormal liver functions before the yeast was removed from the diet, and the dye retention of one was further increased by removing the yeast from the diet. The other three hyperthyroid dogs showed abnormal liver functions when the yeast was removed. These results indicate a relationship between the removal of the yeast from the diet and the production of an abnormal liver function, although the mechanism of such an effect is not clear. It should be noted that the removal of the yeast from the diet of the hyperthyroid dogs was followed by a decline in appetite, which was in turn followed by a secondary loss of weight (11).

Experimental work on the B vitamins has shown (28) that the incidence of hepatic cirrhosis produced in rabbits by lead arsenate was reduced when powdered brewer's yeast was added to the diet. They did not find any relation between the amount of hepatic glycogen and the quantity of arsenic in the liver, or the incidence of cirrhosis. Hirata (17) found that in rabbits with liver dysfunction caused by the injection of chloroform, the administration of vitamin B<sub>1</sub> removed the pronounced decrease in liver glycogen as well as the pathological disturbance, as revealed by the sugar tolerance test. Bartels (3), in a study of the liver function of hyperthyroid patients, found no relation between the duration of the disease and the liver function. He noted, however, that a normal liver function on admission was usually obtained in cases showing an absence of weight loss or a history of no previous iodine administration. The degree of change in the liver function of his patients was in direct relation to the severity of the hyperthyroidism. Similar findings were made by Boyce (5) and by Maddock et al. (19). Maddock et al. reported that 5 toxic patients with an average B.M.R. of +33 per cent had normal hepatic function, whereas 8 toxic patients with an average B.M.R. of +54 per cent showed evidence of hepatic damage. It is possible that the patients cited by Bartels, having a normal liver function and showing an absence of weight loss, were receiving an adequate intake of the B vitamins, but this must remain for future investigation. It is evident, however, from the experimental work reported here, that daily yeast concentrate supplements and vitamin B<sub>1</sub> injections will not reduce the extent of liver damage after it has been produced by thyroid feeding.

Scrutinio has reported (25) that vitamin B<sub>1</sub>, especially intravenous injections, will decrease the basal metabolism and increase the body weight of hyperthyroid patients. Frazier and Ravdin (15) did not find any effect of vitamin B<sub>1</sub>

on the basal metabolism of thyrotoxic patients, but did obtain an increase in body weight under such treatment. The evidence reviewed here certainly demonstrates the need of an increased intake of the B vitamins, particularly vitamin B<sub>1</sub>, during hyperthyroidism, although the rôle that these vitamins play in patients with Graves' disease still remains to be clarified.

*Pulse rate.* The effects of diets, deficient in the B vitamins, on the cardiovascular system of animals and man has been described (15, 29). In the dog and rat a deficiency of vitamin B<sub>1</sub> produces bradycardia, whereas in human beriberi bradycardia is rarely observed and tachycardia is common. As has been pointed out (15), the symptoms of tachycardia, palpitation, dyspnea and fatigability seen in human beriberi are very characteristic of cardiovascular changes seen in patients with Graves' disease. Since a deficiency of vitamin B<sub>1</sub> in humans and hyperthyroidism tend to produce the same cardiovascular symptoms, it has been suggested that a deficiency of vitamin B<sub>1</sub> will intensify the cardiovascular changes produced during hyperthyroidism (4, 15).

In the dog, however, vitamin B<sub>1</sub> deficiency produces a bradycardia whereas thyroid feeding produces a tachycardia. When the dogs were fed thyroid gland the pulse rate rose to an average of 150–160 beats per minute, but the removal of the yeast from the diet after 30 to 60 days caused a marked drop in pulse rate, even while thyroid gland was still being fed. The subcutaneous injection of 2 mgm. of vitamin B<sub>1</sub> in these dogs raised the pulse rate to the previous hyperthyroid within 24–48 hours. Thus the effect of an excess of thyroid gland in producing tachycardia in dogs depends on an adequate supply of vitamin B<sub>1</sub>, although the mechanism of such an effect is unknown. However, after 80 to 100 days of thyroid feeding, the pulse rate falls to 100 to 120 beats per minute and even large amounts of yeast concentrate and vitamin B<sub>1</sub> will not maintain the pulse at higher levels. This pulse rate is slightly above the pulse rate of normal, trained dogs. It appears that the drop in pulse rate at this time interval is associated with a compensatory hypertrophy of the heart.<sup>7</sup>

Frazier and Ravdin (15), in a study on the effects of vitamin B<sub>1</sub> and yeast on the preoperative preparation of the hyperthyroid patient, noted a beneficial effect of the treatment on pulse rate. Bickel (4) has reported that a deficiency of vitamin B<sub>1</sub> aggravates the cardiac disturbances in hyperthyroid patients. From these clinical reports and the experimental work reported, vitamin B<sub>1</sub> seems to play an important rôle in the cardiovascular changes seen during experimental and clinical hyperthyroidism.

#### CONCLUSIONS

1. The results show that a relationship exists between the removal of the yeast from the diet of hyperthyroid dogs and the production of an abnormal liver function.

2. Once an abnormal liver function has been produced, treatment of the hyperthyroid dogs with yeast concentrate and vitamin B<sub>1</sub> injections does not improve the abnormal liver function.

3. The feeding of thyroid gland to dogs produces a marked tachycardia of

150 to 160 beats per minute. The removal of the yeast from the diet after 30 to 60 days causes a marked drop in pulse rate to values below 100 beats per minute, while still feeding thyroid gland.

4: Vitamin B<sub>1</sub> injections into dogs who have had the yeast removed from their diet will raise the pulse rate to the previous hyperthyroid level within 24 to 48 hours. Thus, the action of an excess of thyroid gland in producing tachycardia in dogs depends in some manner on an adequate supply of vitamin B<sub>1</sub>.

5. The rectal temperature of the dogs increased when thyroid was fed, and fell slightly when the yeast was removed from the diet. An increase in rectal temperature was observed when vitamin B<sub>1</sub> was injected.

6. The relation of vitamin B<sub>1</sub> and yeast to the cardiovascular changes and the liver function of hyperthyroid patients is discussed.

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# VANADIUM—A CONSIDERATION OF ITS POSSIBLE BIOLOGICAL RÔLE

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Reports in the literature have indicated a possible biological function of vanadium. Henge (1) showed that vanadium forms 5 to 10 per cent of the respiratory pigment of Ascidians and Phillips (2) and Webb (3) have noted its presence in Holothurians and certain of the molluscs, respectively. Bernheim and Bernheim (4) observed that addition of sodium meta vanadate to rat or guinea pig liver suspensions at pH 6.7 increased oxygen uptake and indicated (5) that the substrate involved was a phospholipid. Attempts to predict the biologically essential elements on the basis of periodic system (6) or atomic structure (7) have included vanadium.

Vanadium has been reported widely distributed in soils (8), plants (9), sea water (10), in deep-sea deposits of the red clay type (11), and in a number of marine products (12). There appear to be differences of opinion regarding its occurrence in milk (13, 14, 15, 16). Although De (17) reported vanadium in human milk, Dingle and Sheldon (18) were unable to detect any trace of this element in either human or cow's milk. Bell (19) did not confirm the findings of Drea (20) who observed in the blood of hens and chicks a concentration of vanadium exceeding the amount found in their feed and suggested a possible hematopoietic function. Keil and Nelson (21) were unable to stimulate hemoglobin regeneration in the rat by the addition of vanadium to an iron-supplemented milk diet deficient in copper. In spectrographic analysis of human tissue, Boyd and De (22) found vanadium in pancreas, kidney, liver, and possibly in spleen but not in brain. Rusoff and Gaddum (23) were unable to detect it spectrographically in the ash of either newborn rats or the stock feed given their mothers.

**EXPERIMENTAL.** These experiments were designed for the purpose of ascertaining the distribution of vanadium in biological materials and to determine whether or not it might be of physiological significance.

Among the materials analyzed for vanadium, eggs and milk were of particular interest, because they represent the sole source of nutrition for the developing chick embryo and suckling young. In addition, materials commonly employed in stock diets for laboratory animals and tissues from normal adult stock rats were analyzed. Samples of muscle, blood, and combined viscera, each sample a composite of similar tissue from several animals, were studied. The ashing of all materials preparatory to spectrographic analysis was carried out by the wet method.

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The spectrograph used for these analyses was a high dispersion, quartz, Littrow type. The arc was operated on a 220 volt D. C. line and a current of approximately 7 to 9 amperes was used. Three types of electrodes were employed, ordinary graphite  $\frac{5}{16}$  inch in diameter which contained slight traces of vanadium, the same electrodes after refluxing in triple-distilled water according to the method of Bell (19) and specially purified, vanadium-free graphite  $\frac{3}{16}$  inch in diameter. For the purpose of testing the sensitivity of the spectrograph for vanadium, spectrograms were made of standard solutions containing 1 to 100 ppm. of vanadium as sodium meta vanadate. In the preparation of these standards the purity of the sodium meta vanadate was determined chemically by both the cupferron gravimetric and the  $\text{KMnO}_4$  volumetric methods. The purity of the sample, found to be 92.28 per cent, was taken into account in

TABLE 1  
*Materials analyzed for the presence of vanadium*

MATERIAL	VANADIUM	MATERIAL*	VANADIUM
$\text{NaCl}$ .....	0	Casein (ordinary commercial).....	0
$\text{Na}_2\text{CO}_3$ .....	0	Casein (Borden's vitamin free).....	0
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ .....	0	Casein (British "Light White").....	0
$\text{MgSO}_4 \cdot 3\text{H}_2\text{O}$ .....	0	Egg white (purchased on open market).....	0
$\text{MgCO}_3$ .....	0	Egg white (from Beltsville).....	0
$\text{K}_2\text{HPO}_4$ .....	0	Egg white (from University of Missouri)...	0
$\text{K}_2\text{CO}_3$ .....	0	Egg yolk (purchased on open market).....	0
$\text{KI}$ .....	0	Egg yolk (from Beltsville).....	0
$\text{K}_2\text{Al}_2(\text{SO}_4)_4$ .....	0	Egg yolk (from University of Missouri)...	0
$\text{CaCO}_3$ .....	v. sl. amt.	Cornstarch.....	0
$\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ .....	0	Whole milk powder.....	0
Ca lactate.....	0	Yeast.....	0
Ferric citrate.....	0	Rat muscle (from normal animals).....	0
Cod-liver oil.....	0	Rat blood (from normal animals).....	0
Butter fat.....	0	Rat viscera (composite sample from normal animals).....	0

\* Materials after first drying in a Freas oven at  $110^\circ\text{C}$ . were moistened with  $\text{HNO}_3$  and heated in silica dishes over a Tirrell burner until a white ash was obtained.

making up the solutions. The most sensitive arc lines of vanadium,  $3183.415 \text{ \AA}$ ,  $3183.99 \text{ \AA}$ , and  $3185.406 \text{ \AA}$ , were considered.

RESULTS AND DISCUSSION. One microgram of vanadium alone on the electrode produced readily discernible spectrum lines; the detection of 0.5 microgram under the same conditions was questionable. If, however, the vanadium solutions were added to 2 mgm. of vanadium-free biological ash on the electrode, as little as 0.02 microgram of vanadium could be quantitatively determined while amounts as low as 0.010 to 0.002 microgram (5 to 1 ppm.) could be detected.

The results of the spectrographic analyses are presented in table 1. All samples failed to show any vanadium except  $\text{CaCO}_3$  which indicated a very slight trace of this element. These findings, contrary to numerous reports in the literature regarding the rather wide distribution of vanadium, bring up a point



which we believe has been overlooked by some investigators in using spectrographic analysis of biological material.

In our earliest experiments, we followed the general practice of using the less pure carbon electrodes known to contain slight traces of vanadium. At that period in spectroscopy, it was considered permissible to use these impure electrodes if control spectrograms were made. Differences between the intensities of vanadium lines produced by the samples and the electrodes alone served as a means of determining roughly the relative amounts of vanadium in the samples. On this basis, vanadium was indicated in considerable quantities in all of the materials tested, with the exception of cod-liver oil and butter fat. Such a method takes no cognizance of the enhancing effect of various elements in the test sample upon the vanadium contained as an impurity in the electrodes.

It is possible under these circumstances to find an enormously greater intensity in the vanadium lines produced by the sample than is found in the blank notwithstanding the absence of vanadium in the sample. Webb and Fearon (6) also experiencing this effect reported that of twenty-two elements commonly occurring in biological material, the enhancing phenomenon was most strongly marked in vanadium, titanium, chromium, and molybdenum. Because this fact has not always been recognized, one is inclined to question the interpretation of many of the reported spectrographic studies. In the case of eggs, for example, it is difficult to believe, in the light of our experiments, that eggs in general contain as much vanadium as Drea (20) has reported. Either Drea's electrodes carried slight traces of vanadium as an impurity or the eggs which he tested are not typical. Those analyzed in this study, in which no detectable vanadium was found, were obtained from three widely different sources: from flocks at the Beltsville Research Center, Beltsville, Maryland, market eggs purchased locally, and a third sample from the experimental flocks at the University of Missouri. The unusually high vanadium values which Bell (19) obtained for eggs, hen tissue, and feed cannot be explained on the basis of contaminated electrodes, since our own impure electrodes subjected to his treatment of purification gave the same results as the special, commercially purified graphite. It would have been of interest had he indicated the ingredients of the chicken feed which conceivably might have carried excessive amounts of vanadium.

It appears significant in the present problem that the eggs secured from the breeding flock at the Beltsville Research Center and shown there to be normal from the standpoint of hatchability contained no demonstrable amounts of vanadium. Such results would indicate that vanadium is not required in any significant quantities for the developing chick embryo. These findings, together with the fact that no trace of this element could be detected in the ration of stock colony rats or in the tissues of animals on this diet, fail to lend support to the idea of a biological need for vanadium. If vanadium functions in such a rôle, it does so in amounts below 1 to 5 parts per million of ashed material. The present study does not exclude the possibility that vanadium may be capable of functioning in place of some other element, phosphorus for example, in cases of an inadequate supply of the latter.

## SUMMARY

Under the conditions used in these experiments, it was possible to detect spectrographically as little as 1 to 5 parts per million of vanadium in the ash of biological materials.

Spectrographic analyses of biological materials do not confirm previous reports that vanadium is rather widely distributed.

The data obtained on the vanadium content of normal rat tissues and egg yolk indicate that vanadium, if it functions in the normal nutrition of the rat or the developing chick embryo, must do so in concentrations of less than 1 to 5 parts per million of the inorganic content.

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# THE EFFECTS OF ADRENALECTOMY AND REPLACEMENT THERAPY ON THE SERUM PROTEIN LEVELS OF THE CAT<sup>1</sup>

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Hypophysectomy produces a marked decrease in serum albumin and an increase in serum globulin concentration in the dog (1) and in the rat (2). The globulin increase appears to be referable to the thyroid deficiency which follows pituitary removal because 1, identical changes can be produced in the rat by thyroidectomy, and 2, administration of desiccated thyroid or thyroxin to hypophysectomized dogs or rats prevents the globulin increase. In such experiments the serum albumin does not appear to be dependent on the level of thyroid activity.

It has been shown indirectly that the activity of the adrenal cortex bears an important relationship to the maintenance of the serum albumin level (2). Administration of adrenocortical substances to hypophysectomized rats to a large extent prevents the decrease in serum albumin concentration. Stimulation of the adrenal cortex of the intact rat by administration of stilbestrol causes a significant increase in serum albumin concentration.

Direct demonstration of the effect of adrenalectomy in the rat was not successful because, as is well known, this species is endowed with accessory cortical tissue in sufficient amounts to maintain life and bodily function for considerable periods. Therefore we transferred our studies to the cat, in which the incidence of accessory cortical tissue is rather low, and studied the effect of adrenalectomy and replacement therapy on the serum protein levels. The results of such experiments, presented in this report, confirm the hypothesis that the maintenance of the serum albumin concentration is related to adrenocortical function.

**EXPERIMENTAL. Methods.** Cats were maintained in the laboratory on a diet of canned salmon, milk and occasionally fresh beef liver. After repeated observations had established that the animals were in good health, blood was drawn and the serum protein levels were determined. Adrenalectomy was then performed. A post-operative interval of at least one week was allowed after the first adrenal was removed and, in most cases, the serum protein levels were determined at this stage. The second gland was then removed. In a few cases, no treatment was given after removal of the second gland until clinical symptoms of cortical insufficiency were apparent and at this time blood samples were obtained and the serum protein levels determined. Most of the animals, however,

<sup>1</sup> Aided by a grant from the Rockefeller Foundation administered by Dr. P. E. Smith.

were maintained by administration of desoxycorticosterone acetate (DCA)<sup>2</sup> or adrenal cortical extract (ACE)<sup>3</sup> for varying intervals until post-operative recovery was complete. Blood was then removed for analysis and the animal allowed to pass into the state of cortical insufficiency whereupon a further blood analysis was obtained. A number of the animals were then restored to health by use of the above mentioned cortical substances and the blood again analyzed. Thus, in several instances, bloods from successive periods of health and cortical insufficiency were obtained. It should be noted that in no case were data for maintenance or restoration used unless the animal either before or after showed the usual clinical and chemical insufficiency syndrome.

Blood samples were obtained from the unanesthetized animals by cardiac puncture. A small portion of each sample was heparinized and used for cell volume (hematocrit) determination. The remainder of the blood was allowed to clot and the serum separated and used for the determinations mentioned below.

Serum protein and non-protein nitrogen levels were determined by methods previously described (2). Serum sodium was determined by the method of Butler and Tuthill (3) and serum potassium by the method of Truszkowski and Zwemer (4).

**RESULTS AND DISCUSSION.** As is now well known (5), adrenocortical insufficiency is associated with well defined changes in the concentrations of certain serum constituents. Sodium is decreased, potassium is increased, urea (or NPN) is increased and there is a pronounced hemoconcentration which is reflected by an increase in the values for hematocrit and total serum protein. In the present experiments (table 1) the usual changes in blood chemistry as well as in clinical condition were encountered when bilaterally adrenalectomized cats were allowed to develop the insufficiency syndrome. On analysis of the serum for albumin and globulin, however, it was found that in every case the apparent increase in total protein concentration was entirely due to the effect of the hemoconcentration on the globulin fraction. In every case of cortical insufficiency, in spite of the increased hematocrit, the serum albumin concentration was found to be at or below the normal level, usually the latter. Such a finding can only mean that during cortical insufficiency, the depletion of serum albumin stores is so rapid that in most cases it is not balanced by the hemoconcentration. Because the concentration of albumin falls while that of the globulin increases, the albumin to globulin ratio is always abnormally low.

If the data for animals suffering insufficiency (table 1, C) is recalculated so as to correct for the hemoconcentration it is found that the total circulating globulin remains substantially at the normal level (3.3 per cent loss) while the total serum albumin stores are depleted by more than 37 per cent. In the absence of

<sup>2</sup> The desoxycorticosterone acetate was generously furnished by Dr. Erwin Schwenk of the Schering Corporation and by Dr. R. C. Mautner of Ciba Pharmaceutical Products, Inc.

<sup>3</sup> The adrenal cortical extract was generously supplied by Dr. David Kline of the Wilson Laboratories and by Dr. G. W. Cartland of the Upjohn Company.

plasma volume studies, such a calculation is based on the assumption that the hemoconcentration is entirely due to loss of water from the plasma. If, as is likely, there is also a coincident decrease in the volume of blood cells, then the albumin depletion is actually greater than the 37 per cent mentioned above.

On restoration of such animals to apparent health or during maintenance after bilateral adrenalectomy the blood chemistry is essentially normal. Such data are shown in table 1, D. The electrolyte and NPN concentrations are within the normal range while the hematocrit value is considerably below normal.

TABLE 1

*Serum constituents of cats before, during and after restoration from adrenocortical insufficiency*

	A NORMAL	B UNILATERAL ADRENALECT.	C BILATERAL ADRENALECT.		E COMPLETE STARVATION FOR 5-12 DAYS
			In insufficiency	Maintained or revived with DCA or ACE	
No. of cats.....	13	10	10	7	2
No. of observations.....	21	11	16*	19†	4
Cell volume %.....	36.2±1.2	32.7±0.8	42.3±1.6	29.2±1.4	37.2±4.0
Potassium, m. eq./l.....	6.02±0.15	5.59±0.46	7.29±0.42	5.35±0.21	5.71±0.36
Sodium, m. eq./l.....	151.3±0.6	151.0±0.6	133.7±1.3	148.8±0.8	142.5±0.8
Non-protein nitrogen, mgm. %	46.0±1.7	46.3±1.9	92.5±9.8	47.1±2.3	40.6±1.7
Total protein %.....	7.58±0.19	7.43±0.11	7.73±0.23	6.88±0.15	7.89±0.22
Albumin %.....	4.01±0.07	3.92±0.12	3.25±0.10	3.45±0.15	4.30±0.06
Globulin %.....	3.57±0.19	3.51±0.09	4.48±0.22	3.43±0.12	3.59±0.28
Albumin Globulin ratio†.....	1.21±0.08	1.13±0.05	0.75±0.04	1.03±0.04	1.22±0.10

± Values are for mean deviation of the mean calculated as  $\epsilon_n = \sqrt{\frac{\sum d^2}{n(n-1)}}$ .

\* 2 without post-operative maintenance; 7 after cessation of 6 to 36 days of post-operative maintenance; 7 after cessation of treatment which restored animal from a previous period of insufficiency.

† 7 after 8 to 24 days post-adrenalectomy maintenance; 12 after restoration from insufficiency by 11 to 43 days of treatment with desoxycorticosterone acetate or adrenal cortical extract.

‡ A/G ratios are the means of individual values rather than ratios of mean albumin to mean globulin.

The latter result is a quite regular feature during DCA therapy and is interpreted as being due to hemodilution, possibly as a result of excessive doses of DCA. The somewhat low total protein and globulin concentrations are in accordance with the hemodilution. In spite of the hemodilution it may be seen that the albumin concentration is increased as compared to the values found during insufficiency. These changes are reflected by the considerable rise of the A/G ratio toward the normal value. If in this case, as above, it be assumed that hematocrit change is entirely due to plasma volume change without alteration of total volume of blood cells, it may be calculated that the corrected value for

the albumin is 4.75 instead of 3.45 per cent while that for the globulin is 4.71 instead of 3.43 per cent.

To illustrate the changes occurring in a single animal a condensed protocol is presented in table 2. In the absence of blood volume studies, it is necessary to interpret the data of this protocol with due regard for the hemodilution resulting each time the animal was restored from insufficiency. Thus, on February 8, 23 days after removal of the second adrenal, maintenance with DCA was discontinued. Twelve days later, on February 20, the animal showed definite indications of insufficiency. Hematocrit and NPN values were increased and

TABLE 2

*Cat 25. Serum changes during adrenocortical insufficiency and DCA restoration*

DATE	BODY WEIGHT	CELL VOLUME	POTASSIUM	SODIUM	NON-PROTEIN NITROGEN	TOTAL PROTEIN	ALBUMIN	GLOBULIN	ALBUMIN GLOBULIN RATIO	
	kgm.	per cent	m.eq./l.	m.eq./l.	mgm. per cent	per cent	per cent	per cent		
1940										
9/16	3.25	34.8	6.35	150.7	47.9	7.25	4.57	2.68	1.72	Normal, no treatment
12/18	4.10	35.4	6.87	150.5	44.8	7.23	4.22	3.01	1.40	Rt. adrenal removed 10/31
1941										
1/27	4.28	34.4	5.52	147.1	42.4	6.79	3.88	2.91	1.33	Left ad. removed 1/17; 10 mgm. DCA daily, appetite good
2/20	3.72	45.1	6.04	136.1	83.9	7.88	3.89	3.99	0.97	Last DCA given 2/8; takes milk but no food last 3 days
3/3	4.42	25.9	3.97	151.4	49.8	6.17	3.43	2.74	1.25	DCA started 2/20; 5-10 mgm. daily, appetite good
3/12	4.34	26.2	5.65	148.5	45.9	7.04	3.80	3.24	1.17	DCA continued; 4-5 mgm. daily, appetite good
3/26	3.94	39.0	7.03	139.5	72.2	8.22	3.84	4.38	0.88	Last DCA given 3/14; takes milk but no food last 2 days
4/24	4.45	26.7	4.65	149.2	42.6	6.87	3.68	3.19	1.15	DCA started 4/1; 6 mgm. daily, appetite good
5/7	3.93	40.8	7.24	135.5	88.0	8.05	3.89	4.16	0.94	Last DCA 4/26; takes little milk and no food last 4 days

sodium was low. Total protein was increased but this was entirely due to the globulin increase. The albumin value was identical with that found during maintenance of health with DCA. This means that serum albumin actually decreased but was compensated by the hemoconcentration. The A/G ratio, as expected, was considerably below the normal value for this animal. Exactly the same situations are seen in the two subsequent periods of insufficiency on March 26 and on May 7.

The effects of restoration from insufficiency by injection of DCA are shown by the data for March 3 and for April 24. In each instance the serum sodium, potassium and NPN were restored to the normal levels. The hematocrit value

was reduced, indicating a marked hemodilution which was reflected by the decrease in total protein (and globulin) concentration. In the case of this cat, the albumin also fell slightly during these two periods of restoration but this is obviously due to the marked hemodilution. Although the hematocrit value decreased to almost half the value observed during insufficiency, indicating an increase in plasma volume to two and one-half times its former value, the albumin concentration decreased by a very small amount (12 and 4 per cent). The reciprocal nature of the changes in albumin and globulin concentrations is definitely shown by the marked increase in A/G ratio during each restoration.

It is of some interest that after removal of the first adrenal gland, the albumin concentration fell while that of globulin increased in a fashion similar to that observed during insufficiency (table 2). This phenomenon was frequently seen even though at such times the cats appeared to be in excellent health. In several instances in which additional blood samples were taken before removal of the second gland, it was found that there was a gradual return of the protein concentrations toward the preoperative levels. We have interpreted this to mean that on removal of the first adrenal the animal was temporarily thrown into a mild subclinical hypocortical state and that if sufficient time were allowed for hypertrophy of the remaining gland, a gradual return to an entirely normal state resulted.

It will be noted (table 2) that during each period of insufficiency cat 25 lost from 7 to 13 per cent of its body weight. A large proportion of this weight loss is probably due to dehydration. Another portion is probably a result of decreased food intake during the last 2 or 3 days of the acute insufficiency. Although we have shown (2) that in the rat inanition for as long as 3 weeks has but a slight effect on the blood protein picture, it was thought advisable to check this point in the cat. For this purpose two cats were completely starved, each for two periods. The duration of starvation ranged from 5 to 12 days. The data obtained after complete starvation is given in table 1, E. There was in no case any noteworthy change in either albumin or globulin concentration or in A/G ratio. The only change noted was a slight decrease in serum sodium concentration after the longer periods of inanition. It may be of interest to note that both adrenals had been removed from each of these animals prior to the starvation experiments. Because insufficiency did not develop it was assumed that accessory cortical tissue was present. Such an accessory was later removed from the region of the spermatic artery of one of these cats with the prompt appearance of insufficiency symptoms.

Weech (6) has adequately demonstrated the importance of protein deprivation with regard to maintenance of the serum albumin concentration. It appears, however, that the periods of inanition reported here were too short to show the effects noted by Weech. Because the inanition during the periods of complete starvation was much more severe than that suffered by the animals during insufficiency, it becomes evident that during the latter state the changes in serum albumin concentration are due to deranged metabolism rather than to simple inanition.

In the cat, as in the rat, serum globulin metabolism appears to be independent of the level of adrenocortical activity and apparently proceeds normally even during rather severe insufficiency. The total globulin stores remain more or less constant although hemoconcentration causes a proportional increase in the globulin concentration which entirely accounts for the frequently reported rise in total protein level.

Albumin replacement appears to be considerably impaired during adrenal insufficiency as evidenced by the considerable loss of this substance from the blood. This loss is of such a magnitude that it is not balanced by the hemoconcentration with the result that the concentration of the albumin usually falls in spite of the increasing hematocrit. These findings are, therefore, in good agreement with our earlier work (2) which indicated that in the rat maintenance of a normal globulin level is related to the degree of thyroid activity while serum albumin replacement is dependent on adrenocortical function.

The findings reported here are also in accord with the present knowledge concerning the effect of the adrenal cortex on general protein metabolism. According to Long (7, 8) the action of the cortical hormones favors catabolism of body protein to amino acids and conversion of a portion of these to carbohydrate. It is not inconsistent with this theory that another portion of such newly formed amino acids may be used in the synthesis of vitally important proteins such as serum albumin.

It is well known that the serum albumin is of major importance in the maintenance of the osmotic relationships of the blood and therefore of the blood volume. Equally widely accepted is the concept of control of blood volume by the hormonal influences of the adrenal cortex. The concept offered in this paper appears to provide a missing link relating these two facts although it remains for further investigation to show whether blood volume change precedes albumin change or vice versa.

The authors wish to acknowledge the important technical assistance of Mrs. Elizabeth Wolfe.

#### SUMMARY

The well known increase in the concentration of total protein in the serum of adrenalectomized cats is entirely due to an increase in the globulin level. Despite the pronounced hemoconcentration, the serum albumin concentration decreases or remains at the normal level, indicating marked loss of albumin from the circulatory system.

Restoration of cats from adrenal insufficiency to health by means of desoxycorticosterone acetate or adrenal cortical extract produces hemodilution and corresponding decrease in the levels of serum globulin and total protein but the serum albumin concentration increases.

Because the serum albumin and globulin change in opposite directions, the A/G ratio falls markedly during insufficiency and increases when replacement therapy is administered.



From these findings and from previous results obtained from rats, it is concluded that in these species the adrenal cortex exercises a positive control over the metabolism of serum albumin but not of serum globulin.

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# STUDIES ON IRRADIATED CEREBRAL DIFFERENTIATED EXCITATION AND INHIBITION AS INDICATED AND MEASURED BY RESPIRATION<sup>1</sup>

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While correct conditioned differentiation is obviously dependent on correct discrimination, this problem is not concerned with fine discriminations of the analysers used, but rather with the finesse of the reflex. Correct conditioned differentiation then signifies that either reflex, positive or negative, must be correct irrespective of any order or sequence of the two tests or any time interval between the tests, which varied from the usual duration of about 2 minutes to a 2 second interval.

Flexion of the right foreleg was chosen for the motor response, using a modification of Beritoff's procedure. It should, however, be emphatically stated that reinforcement was never used except for punishment of errors. In developing the positive conditioned reflex we rewarded the correct responses and punished the errors. For a positive conditioned reflex to be correct the dog must flex the foreleg within 7 or 8 seconds, which would break a switch and prevent an electric shock on the same foreleg. To obtain correct conditioned differentiation we varied somewhat the technique of Pavlov and Beritoff. Instead of rewarding or punishing only the positive conditioned tests, we punished the errors for a positive test by shock and the errors for a negative test by whipping or scolding depending on the temperament of the dog. Under this procedure a positive conditioned reflex ordinarily became well established within 25 trials, sometimes appearing on the second trial and when once acquired rarely had to be reinforced by an unconditioned stimulus. The time in which correct conditioned differentiation was obtained varied considerably—differing in dogs, in the closeness of discrimination required and for the analyser used. It is well-known that dogs do not use optic sense to the extent that they use olfaction and hearing, hence the difficulties to be noted later for this analyser in correct conditioned differentiation.

*Effect of positive and negative conditioned reflexes on cerebral potentials.* This, the first method utilized, consisted of taking simultaneous oscillograph records from the surface of the brain (motor foreleg center, a more median area VI? or the prefrontal area) and from the foreleg musculature at the time of a positive or negative conditioned foreleg reflex. The brain electrode, a home made instrument, was previously implanted and firmly fastened to the skull. Some records taken 12, 24, 36, 48 and 60 hours after implantation of the electrode, revealed suggestive changes in the Berger waves, but since similar changes also occurred during the intervals between tests this procedure was replaced by another.

<sup>1</sup> Aided by a grant from the John and Mary R. Markle Foundation.

RESPIRATORY MEASUREMENT OF IRRADIATED CEREBRAL EXCITATION AND INHIBITION DURING CONDITIONED DIFFERENTIAL TESTS. This mode of approach was suggested (1929 and 1936) by the accuracy with which olfactory, trigeminal and vagal subcortical reflexes could be analyzed by respiration and by the ease with which cortical dominance would alter them. The method consists of taking kymograph tracings of thoracic respiration at the time of recording positive and negative conditioned reflexes. Attention is called to the fact that the signal for introducing a conditioned test is not always perfectly timed as there may be some variation in the receipt of the stimulus. This is especially true for the general cutaneous and olfactory analysers. In addition to the controls described for earlier work, respiratory tracings were recorded before conditioning, so that the characteristics of a subcortical reflex for any analyser are well known. All tests were made before feeding and the room temperature was maintained approximately the same. Careful notes were taken of the animal's behavior during the tests.

Some advantages of this method are that the respiratory records show: 1, the exact time of appearance and disappearance of the irradiated excitation and inhibition in the motor cortex (respiratory area); 2, the manner of their appearance and disappearance; 3, their relative strengths. In addition one can always see on the drum what is taking place and can select a time for stimulating, when the animal is quiet and breathing normally. Most important, however, is the extreme sensitivity of the cortical respiratory area to the irradiated excitation and inhibition which accompany a conditioned reflex. Ordinarily there is no difficulty in interpreting a thoracic respiratory tracing as to excitability or inhibition. An occasional record, however, if considered by itself, might be confusing. It is not assumed that irradiated excitation or inhibition is the same throughout the entire motor cortex or that it might not vary in different areas with different tests. It is also evident that excitatory and inhibitory changes are taking place occasionally between tests from anticipation, external or internal stimuli. Usually under the conditions of our experiments respiration will continue regularly in most dogs for long intervals of time.

*Auditory analysers.* The tapping of a bell and a board once per second for a 7 second interval served as positive and negative stimuli for the first conditioned differential foreleg tests with this analyser. For descriptive purposes liberal use is made of data gathered from an active, easily inhibited shepherd dog 1. Before conditioning was attempted for this dog, the first sound of the bell elicited a slight depression of the first two respiratory excursions, due possibly to the dog's coming to attention. Later tests, third bell and second board (fig. 1, A and B) showed little or no change in respiration.

It sometimes happens in auditory and olfactory conditioned differential tests that the first negative conditioned test is correct. It has been a matter of speculation whether this absence of response signifies that the antagonistic stimuli (bell versus board and cloves versus asafetida) are so widely separated as to tone and odor that the first trial of the negative stimulus failed to elicit cerebral activity or whether the dissimilar negative stimulus furnished the re-

quired inhibition for the correct reflex. A possible answer to this question is found in the respiratory tracing of the first negative test (fig. 1, C), which shows inhibition both in amplitude and rate.

Records D to F of figure 1 were taken from dog 1 after correct auditory conditioned differentiation was well-established. Record D shows a correct positive

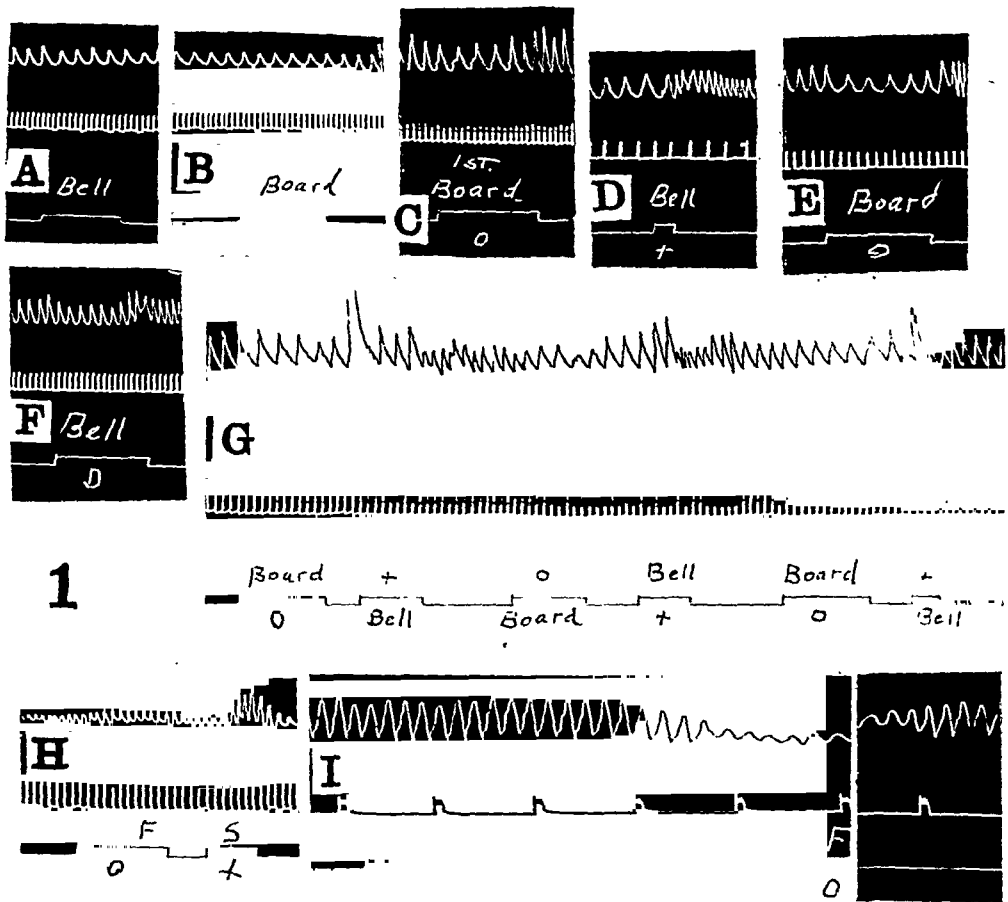


Fig. 1. Top to bottom—thoracic respiratory tracing, upstroke = inhalation; time in seconds; conditioned reflex, upstroke = beginning of test and downstroke = response or end of test (positive foreleg response indicated by + and negative 0). A to G sound tests dog 1 (Bell = positive and Board = negative). A and B, before conditioning; C, first negative test; D to F, after conditioning; G, negative and positive tests alternated in quick succession. H to I, sound tests (S = slow bell, positive and F = fast bell, negative); H, negative and positive tests changed in quick succession (dog 5); I, small portion of a negative test (easily inhibited dog 3), space represents a 14 second interval.

conditioned response of the foreleg to the bell within 1 second and acceleration of respiration accompanied by an expansion of the thorax; while record E portrays a correct negative conditioned reflex of the foreleg together with marked inhibition of respiration (decrease in height and rate of excursions) during a 10 second interval of tapping a board. In both instances respiration was normal at first and returned to normal within 10 seconds after completing the test.

In figure E note the rebound (positive induction) at the termination of the wave of inhibition. Record F of figure 1 is presumably to be interpreted as an example of incorrect spread of cortical inhibition, since it demonstrates that failure of the auditory conditioned foreleg response is accompanied by inhibition of respiration. Dog 1 made very few errors with his negative auditory tests and no respiratory tracings are available.

After correct differentiation had been established for dog 1, the period of inhibition accompanying a negative test continued for no longer than 20 seconds. The period of excitation was usually much shorter, often only a few seconds' duration. Both appeared and disappeared abruptly.

Dog 2, a very excitable animal of the fox terrier variety, produced respiratory tracings very similar to those of dog 1 during the auditory conditioned tests. In addition to the above mentioned tests for dog 1, several long series of alternate positive and negative conditioned tests, including respiratory tracings, were made within intervals of 2 to 10 seconds to determine if the excitatory or inhibitory state from the previous conditioned reflex produced any pronounced effect on the following test. A representative series (fig. 1 G) demonstrates that the cortical effects on the foreleg and respiratory musculature were practically instantaneous and that the conditioned stimulus, positive or negative, was very effective on a presumably excited or inhibited motor cortical area, not only for one, but for a great many auditory conditioned reflexes.

Dog 3, a bright, but very easily inhibited animal,<sup>2</sup> was of especial interest on account of a 20 second interval of pronounced inhibition of respiration (identical to the partial record of figure 1, I), which accompanied a correct negative auditory conditioned test. The wave appeared within 1 to 4 seconds after the test started and continued for 10 to 20 seconds after the test ended. The incorrect negative conditioned tests for this dog were also of interest in that they demonstrated excitation of respiration during the first few seconds of the test in the form of increased height of the excursions, which changed in the later seconds of the test to a marked and long continued period of inhibition as was characteristic of the correct negative conditioned tests.

Dog 4, a springer spaniel and dog 5, a mongrel, both bright, active, and possessing well balanced cortical activity (neutral type of Pavlov) disclosed slight or no inhibition of respiration during their correct negative tests with the board (fig. 2, A). On the other hand, it is significant that positive foreleg responses to the bell and all incorrect negative responses to the board (fig. 2, B) ordinarily were accompanied by considerable excitation of respiration. As for the control respiratory tests for the bell and board before conditioning, all but the first

<sup>2</sup> As illustrative of the ease with which dog 3 could be inhibited, it became necessary to whip this dog for becoming unmanageable during the tests with the positive conditioned reflex for cloves, a test used for determining the dog's fitness for conditioned differentiation. Afterward it required over 50 tests, spread over 2 days, to evoke a conditioned foreleg response. The dog behaved like a balky horse, hung loose in harness, made no attempt to cooperate in the tests and took shock after shock without a whimper for failure to flex foreleg. We afterward learned that this dog was so easily inhibited that the word "shame" furnished the required inhibition to suppress the foreleg response in developing the negative conditioned reflex.

(the majority of which showed inhibition and a few excitations) revealed unchanged respiration during or following the sound stimulations.

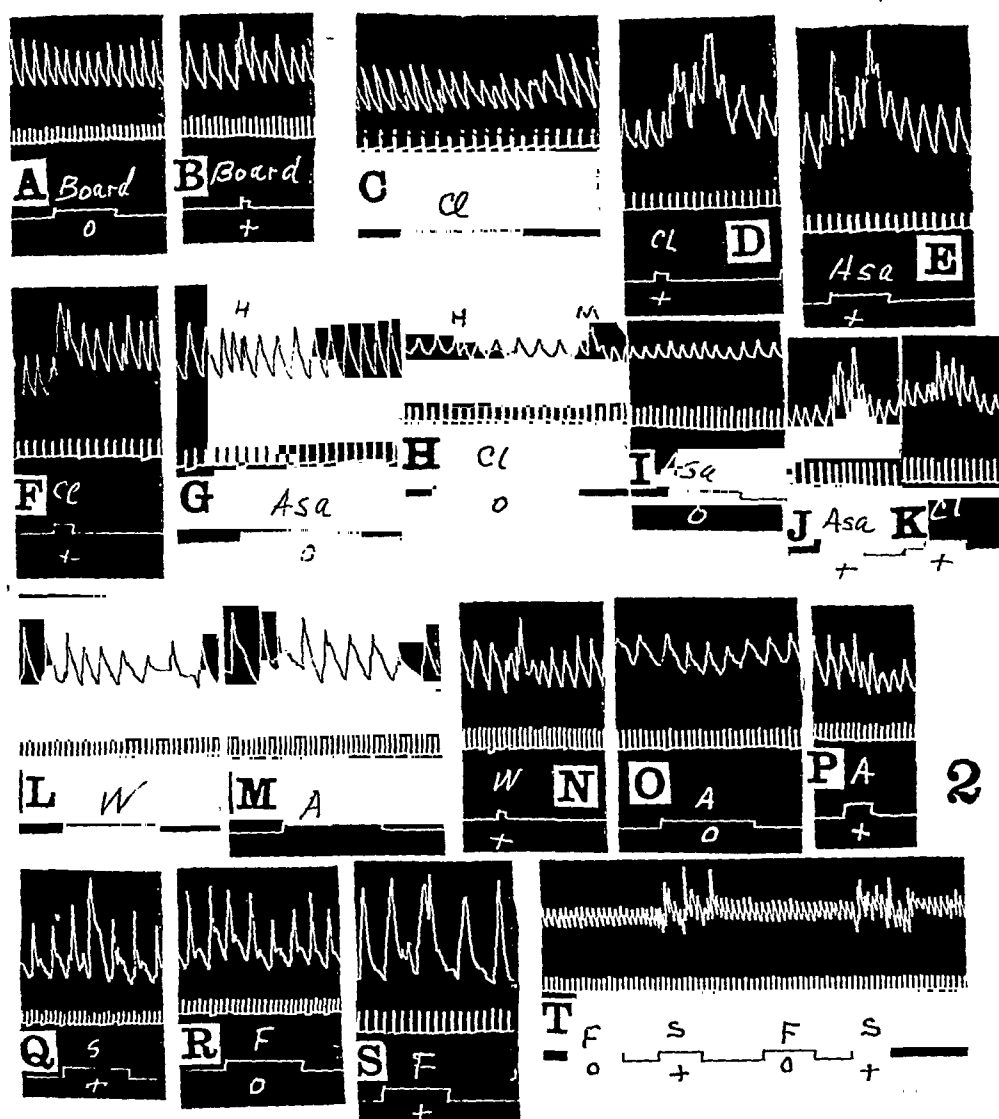


Fig. 2. Top to bottom—thoracic respiratory tracing, time in seconds, conditioned responses (indicated + or 0). A and B, negative sound tests (dog 4) C to K olfactory tests (Cl = cloves, positive and Asa = asafetida, negative) dogs 4 and 5; C, before conditioning; D and E, during differentiation; F and G after differentiation; H to K, as above, dog 5. L and P, general cutaneous tests, dog 2 (W = with grain of hair, positive and A = against grain, negative); L and M, before conditioning; N to P, after differentiation. Q to T general cutaneous tests (S = slow brush movement, positive and F = fast, negative) after differentiation; Q and R from dog 2 and S, dog 4; T, dog 5, negative and positive tests alternated within a few seconds.

Respiratory tracings were recorded from 2 of our 5 test dogs for a second auditory conditioned differential test based on rate of the same sound. A bell tapped once per second and three times per second served for positive and negative conditioned tests.

After conditioned differentiation was established for the neutral dog 5, record H (fig. 1) discloses the ability of this dog to respond correctly to a positive conditioned reflex (1 bell per sec.) immediately after having responded correctly for the negative test (3 bells per sec.). It will be seen that respiration was changed from slight inhibition in the first test to marked excitation in the second.

The respiratory tracings from the easily inhibited dog 3 were also of interest in the second series of auditory conditioned tests on account of the marked inhibition that occurred in the negative tests. This started in 1 to 3 seconds and continued for 10 to 20 seconds after the test ended. A portion of one of these tracings recorded on a fast moving drum (fig. 1, I) reveals marked inhibition of respiration starting rather abruptly after 3 seconds of the test had elapsed and an abrupt return to normal. The break represents a 14 second interval, the first 3 seconds of which were included in the test. The respiratory tracing during an incorrect negative conditioned test frequently shows a brief interval of higher excursions, followed too late by period of marked inhibition. Obviously the fast breathing of this dog was not favorable for further acceleration during a correct positive test, but its depth was generally increased.

*Olfactory analyser.* As in previous studies, clove vapor was used for positive conditioning and asafetida for negative. It is well known that insufflation or inhalation of purely olfactory stimulating vapors produce, subcortically, instantaneous and pronounced inhibition of respiration (fig. 2, C and 1929 paper fig. 3, E). One excitable dog and two of the neutral type were used with this analyser. Since all tracings used in the text are from the neutral dogs they will exhibit less alteration of respiration.

Thoracic respiratory tracings recorded from dog 4 during the early tests for development of correct conditioned differentiation when positive responses of the foreleg were common for asafetida, demonstrate very similar tracings for both vapors (fig. 2, D and E). These tracings disclose marked excitation, consisting of an immediate expansion of the thorax starting on the exhaling phase and followed by stronger accelerated excursions. After the tests respiration rapidly approached normal. When correct conditioned differentiation was established, the correct positive reflex tests (fig. 2, F) ordinarily showed a less amount of excitability than before; while the respiratory tracings recorded during a correct negative conditioned reflex test (fig. 2, G) revealed slight, but usually some inhibition. It was characteristic of this and other dogs after taking a whiff of a vapor to turn head in opposite direction. This movement is accompanied by an extra respiratory excursion (fig. 2, G and H). Olfactory conditioned differentiation became so perfect with this dog that no incorrect positive or negative conditioned reflexes were recorded.

Dog 5 like dog 4 had a perfectly balanced cerebral cortex, and the respiratory tracings recorded during incorrect positive and correct negative olfactory conditioned tests (fig. 2, H and I) exhibit little or no inhibition. These tracings are in every way comparable to similar records taken from this dog with the auditory analyser. Movements of the head or body during a negative reflex test elicit slight excitation of respiration (H and M, previous figure). These

effects on respiration are seldom comparable to the more pronounced excitation which accompanies an incorrect negative and a correct positive conditioned reflex (fig. 2, J and K).

After correct olfactory conditioned differentiation was well-established for the excitable dog 2, the respiratory tracings recorded during correct positive and incorrect negative conditioned reflex tests revealed marked excitability and the correct negative and incorrect positive conditioned tests disclosed fully as much inhibition of respiration as was exhibited by the previous auditory conditioned tests for this dog.

*General cutaneous analyser.* Two different types of conditioned differentiation were used for this analyser. 1. Consisted of stroking the hair on the back with a handbrush once per second with the grain for the positive reflex (indicated by W in the records) and against the grain once per second for the negative reflex (A, in records). 2. Consisted of stroking the back once per second with the grain as for the previous positive test (S, in records) and 3 strokes per second with the grain for the negative reflex (F, in records). It should be noted that the stimuli used to elicit the negative conditioned reflexes were those which presumably would evoke more excitability of respiration subcortically than the stimuli used for the positive reflexes. One excitable and two neutral dogs were used for these tests. All tracings but the last were recorded from the excitable fox terrier 2.

Respiratory tracings recorded before conditioning during many control tests with the positive and negative general cutaneous stimuli demonstrated considerable variation of effect upon respiration. The first and second application of the positive stimulus on dog 2 evoked inhibition, the third and fourth, excitation; while the fifth positive and the first negative stimulus (fig. 2, L and M) produced slight depression. During the early stages of the differential tests, when errors were common for both tests, the positive foreleg responses were accompanied by considerable excitation of respiration; while the absence of foreleg or other movement during either test disclosed marked inhibition of respiration. After differentiation was established the respiratory tracings recorded during correct positive and negative conditioned tests (fig. 2, N and O) still revealed excitation or inhibition. Occasionally at the time of the first application of the brush or some body movement, the negative conditioned tracings would show a brief interval of excitation of respiration. This change in respiration was rarely comparable to the change which accompanied a conditioned foreleg response. The respiratory tracings accompanying all incorrect negative conditioned tests revealed excitation (fig. 2, P). There were practically no errors for the positive conditioned-tests.

The tracings for the excitable dog 2 are also fairly representative for the two dogs of the neutral type, except that they show every little or no inhibition during a correct negative conditioned test.

In the second method used for obtaining general cutaneous differentiation where a slow stroke of the brush indicated a positive conditioned reflex and a fast stroke, a negative reflex, all correct positive and negative conditioned reflex



tests (fig. 2, Q and R) were ordinarily accompanied by excitation and inhibition of respiration with the excitable dog 2. As noted for the previous differential method there were brief intervals of slight excitation of respiration approximating the first touch of the brush during some correct negative conditioned tests.

The two dogs of the neutral type revealed little or no inhibition of respiration during the correct negative conditioned tests, but inhibition was always present in some tracings. On the other hand, the few incorrect negative conditioned reflex tests (fig. 2, S) always showed some excitation of respiration.

Record T (fig. 2) demonstrates how rapidly dog 5 could make a correct change from one reflex to the other. As little as a 5 second interval after a correct negative conditioned reflex, showed no effect of the previous irradiated wave of inhibition on the following positive conditioned reflex. The respiratory tracing discloses considerable excitation during the positive tests and little or none during the negative tests.

A comparison of the respiratory tracings recorded during the conditioned reflex tests by both methods of obtaining general cutaneous differentiation with similar tracings of the auditory and olfactory analysers after conditioned differentiation had been acquired, discloses similar but less alteration of respiration.

*Optic analyser.* All tests were made by flashing a diffused light on painted wooden screens (10 by 14 in.) placed during darkness on a black box in front and to one side of the dog, the dog being fastened so that he must at all times look at the screen. The light was turned on from the adjacent room when animal was quiet and breathing normally. The positive conditioned test lasted for 7 seconds and the negative from 8 to 15, depending on the time of the appearance of the positive foreleg responses. All tests were preceded by an interval of absolute darkness, during which the screens were changed.

Three different conditioned differential tests were used for this analyser. 1. White and black screens were used for the positive and negative tests (L and B in tracings). 2. A constant light and a flicker of 3 flashes per second on a white screen served for the positive and negative conditioned reflexes (L and F in tracings). 3. A white circle and cross on black backgrounds served for positive and negative conditioned reflexes (Cir and Cr in tracings).

Control records of the effects of these optic tests on respiration before conditioning revealed no change except during a few of the first tests.

Of the 3 dogs used in the white (positive) and black (negative) conditioned reflex tests the two neutral dogs 4 and 5 learned to differentiate fairly well, but the excitable dog 2 was extremely variable. Throughout these tests the dogs demonstrated their mental difficulties as follows: During a correct negative conditioned test with black, the neutral dog 5 would frequently flex the opposite foreleg and make other body movements. The excitable dog 2 developed a series (2 to 6) of very weak pulls, which might or might not sum to break the switch within the time of the test.

Respiratory tracings recorded during correct positive or incorrect negative conditioned tests for the neutral dog 5 (fig. 3, A and B) disclose about the same

amount of excitation as was demonstrated for the previous analysers. Likewise a correct negative conditioned reflex test (fig. 3, C) reveals but slight inhibition or no change in respiration. The respiratory changes for the other neutral dog 4 were similar to dog 5. The excitable dog 2 when differentiating correctly, demonstrated marked inhibition of respiration during his correct negative conditioned tests. Record D (fig. 3) shows the occasional ability of this dog to make correct negative and positive conditioned reflexes to black and

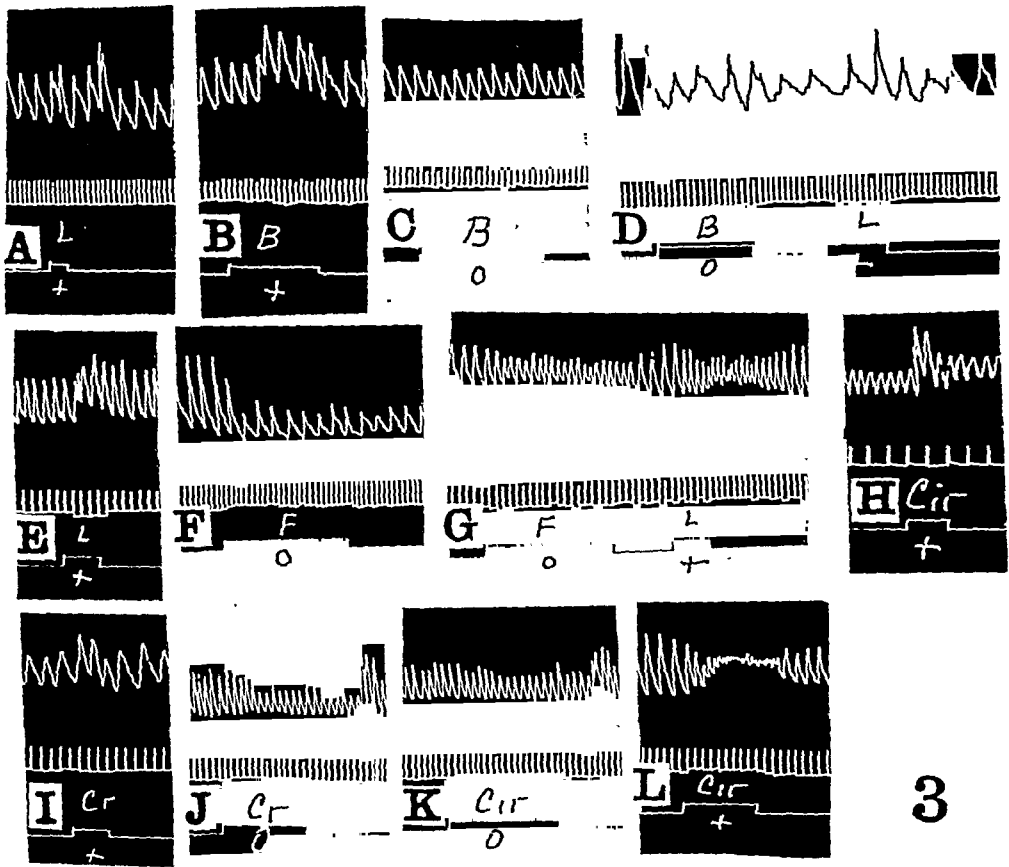


Fig. 3. Top to bottom—thoracic respiratory tracing, time in seconds, conditioned responses (indicated + or 0). A to D light tests (L = white, positive and B = black, negative); A to C from dog 5 and D, dog 2. E to G, light tests (L = constant, positive and F = flicker, negative); E from dog 5; F, dog 2 and G, dog 4. H to L, light tests (Cir = circle, positive and Cr = cross, negative); H to K from dog 5 and L, dog 4.

white, together with the corresponding changes in respiration, when the intervals between these two tests are only a few seconds apart.

As for the constant light and flicker conditioned differential tests, the excitable dog 2 and the neutral dog 5 acquired differentiation to a considerable degree of accuracy. A typical respiratory tracing from dog 5 recorded during a correct positive conditioned test (fig. 3, E) shows excitability of respiration, while a similar record from the excitable dog 2 during a correct negative flicker test (fig. 3, F) discloses the marked inhibition of respiration that was characteristic

of the dog for other analysers. It should be noted for dog 5 that some of the respiratory tracings recorded during correct positive conditioned reflex tests, in which the foreleg response was late in appearing, revealed a short interval of inhibition at the beginning of the test, which soon changed to excitation. It may be added that the respiratory record obtained during a correct negative conditioned reflex test from dog 5 revealed slight or no inhibition.

The other neutral dog 4 never acquired the ability correctly to differentiate conditionally between a constant light and a flicker. He would respond correctly to either in a series, but with an abrupt change, errors were the rule. After 700 tests, a preponderance of positive tests over negative tests resulted in nearly all tests going positive, and with the reverse all would go negative. It is of especial interest to note that during this dog's confusion, the foreleg response became altered from a quick upward jerk to a slow sidewise movement that required 2 to 6 seconds to break the switch. This type of pull persisted for all responses, correct or incorrect. The respiratory tracings were identical for all tests, the excursions disclosing marked depression and acceleration (fig. 3, G). In this tracing the negative and positive tests were given in quick succession and happened to be correct. The depression of the excursions was usually much more pronounced in the single tests than it was in this combination.

None of the three dogs used with the circle for positive and the cross for negative conditioned tests could be said to differentiate correctly conditionally. The neutral dog 5 made the best record. Out of a total of 1168 tests 377 positive tests were correct and 117 incorrect and for the negative tests 382 were correct and 292 were incorrect. With the other analysers, this dog would average better than 95 per cent correct.

The neutral dog 5 never became excitable during these tests and all respiratory tracings were true to form (fig. 3, H to K). In these records the correct positives and incorrect negative tests showed excitation of respiration, while the correct negatives and incorrect positives exhibited slight or no inhibition. During a correct negative conditioned reflex test there was often a flexion of the opposite foreleg.

During the first 300 tests with the circle and cross the neutral dog 4 behaved very much as he did with the previous optic differential tests. All foreleg responses consisted of the same very slow sideward pull and the respiratory tracings (fig. 3, L) demonstrated pronounced depression and some acceleration. From this time on, the dog went off on a tangent. All of his differential tests were either positive or negative, depending on which predominated. If the positive tests were more numerous the dog became generally excited, frequently flexing his foreleg between tests. In these tests the respiratory tracings portrayed excitability and the deliberate foreleg flexion of the earlier tests changed to a quick pull. With a predominance of negative tests, the animal was quiet and respiration generally showed slight inhibition or no change during a test. After completing a day's tests and showing no sign of differentiation with the circle and cross, this dog was always able to differentiate correctly with the foreleg and respiration during positive and negative auditory conditioned tests.

The excitable dog 2 became hopelessly confused with the circle and cross conditioned differential tests and became fully as neurotic as Shenger-Krestovnikova's dog did with the elliptical tests, often terminating an experiment with a spasm. Upon completing a day's tests, this dog was unable to respond correctly to positive and negative auditory conditioned tests.

**DISCUSSION.** The presence of excitatory and inhibitory cerebral areas is well known from the investigations of Adrian, Dusser de Barenne et al., Rioch and Rosenblueth, Tower, McCulloch, Walker and others.

Particular attention is called to: 1. Ability of these dogs to respond correctly to a long series of quick alternate changes from negative to positive conditioned tests and the reverse when respiration was in a state of inhibition or excitation from the previous test. 2. The respiratory changes during the process of obtaining correct olfactory conditioned differentiation—where the unconditioned inhibitory reflex was changed to excitation during a correct positive conditioned test and finally to one of excitation or inhibition during a correct positive or negative differential test. 3. Significance that was attached to a first auditory and other first negative conditioned tests being correct and showing inhibition of respiration. 4. Alterations of the characteristic conditioned foreleg response and corresponding respiratory changes which resulted from the inability of the dogs to differentiate perfectly with the optic analyser.

The fact that inhibition of respiration is often absent during many correct negative conditioned tests with the neutral type of dog may be construed to mean absence of irradiated suppression in the respiratory center. On the other hand, since inhibition of respiration occurs during some correct negative conditioned reflex tests with the neutral dog and excitation of respiration usually accompanies an incorrect negative conditioned reflex, the question might be asked—is there not some irradiated inhibition or some form of suppression present in the respiratory cortex as well as in the foreleg cortex to keep respiration unchanged during a correct negative conditioned test?

This study not only supports Pavlov's deductions that cortical excitation and inhibition are most important in the formation of positive and negative conditioned reflexes, but they are also essential for obtaining and maintaining correct conditioned differentiation. The immediate appearance and short duration of the irradiated excitation or inhibition of respiration during a correct positive or negative conditioned reflex test is favorable to Beritoff's contention that the excitation associated with a conditioned reflex originates from a direct focus and lasts only as long as the excitation lasts. The instantaneous inhibition of our conditioned differential tests is not comparable to the slow and prolonged inhibition reported by Petrova and others.

The correct irradiated excitation and inhibition accompanying our correct conditioned differential tests suggests the presence of groups of association cells in cerebrum, switchmen, which may suppress the motor cortex or impulses going to it until a decision is reached from previous experiences and failures with the stimuli used in the tests (associated memory) and then there are released impulses which may fire or suppress motor activity.

After conditioned differentiation was perfectly established by our procedure there was apparently more than a temporary connection from the analyser center to the association cells to the motor cortex. These connections functioned perfectly and with undiminished strength after being unused by these reflexes for 6 months.

#### SUMMARY AND CONCLUSIONS

Control tests before conditioning produced subcortically from olfactory stimulation—inhibition of respiration; from general cutaneous stimulation—either inhibition or excitation; from auditory or optic stimulation—no change except for the first few tests.

Thoracic respiration showed the following changes after conditioned differentiation had been established with auditory, olfactory, general cutaneous and optic analysers: 1. A correct positive or an incorrect negative conditioned test demonstrated excitation in all dogs. 2. A correct negative or incorrect positive conditioned test exhibited marked inhibition with the excitable or easily inhibited dog, while the evenly balanced or neutral dog disclosed slight or no inhibition.

A series of alternate negative and positive conditioned foreleg reflexes and their corresponding changes of respiration may occur correctly when the tests are only a few seconds apart and when respiration (motor area) is still in a state of inhibition or excitation from the previous test.

A possible contest for supremacy is suggested by the presence of waves of excitation and inhibition, correct and incorrect, in the respiratory records of some of the dogs which had difficulty in responding correctly to the differential tests.

Two or more responses of the foreleg during a positive conditioned test were usually accompanied by separate waves of excitation of respiration or intensifications of the common wave. Termination of the inhibitory or excitatory effect on respiration was often followed by a rebound. Likewise the excitable dog upon finishing a correct negative test frequently flexed his foreleg.

There are apparently 3 kinds of instantaneous cerebral excitation and inhibition (suppression). 1, direct to lower areas; 2, incorrect; 3, correct.

The selection of respiration as the mechanism for indicating and measuring the irradiated cerebral excitation and inhibition accompanying the positive and negative foreleg reflexes utilized in correct conditioned differentiation appears to be well justified by the results of this study. It is apparent for dogs that this high order of excitation and suppression takes priority over all others.

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## INDEX

- A**BSORPTION and disposition of glucose in chick, 244.
- , fat, adrenalectomy and, 712.
- , intestinal, of chloride, bile salts and, 340.
- Accommodation in mammalian motor nerves, 629.
- Acetylcholine, action of, on cardiac ganglion of *Limulus*, 182.
- Acid-neurohumoral mechanism of nerve cell activation, 604.
- Acidity, duodenal, fat ingestion and, 285.
- Action potentials in visceral smooth muscle, 553.
- Activity and food intake after hypothalamic lesions, 609.
- ADOLPH, E. F. See PINSON and ADOLPH, 105.
- Adrenal cortex, pituitary and, in resistance to cold, 22.
- steroids in circulatory failure, 567.
- Adrenalectomy and fat absorption, 712.
- , renal function after, 229.
- Adrenalin and stimulation of hypothalamus, 376.
- , circulatory responses to small amounts of, 87.
- Adrenals and serum protein levels, 776.
- , relation of, to serum albumin and globulin levels, 306.
- ALLEN, W. F. Studies on irradiated cerebral differentiated excitation and inhibition as indicated and measured by respiration, 783.
- ALLES, G. A. and G. A. FEIGEN. The influence of benzedrine on work-decrement and patellar reflex, 392.
- Alpha waves, pulse and, of tips of fingers and toes, 448.
- Altitude, high, sulfanilamide and, 494.
- Amniotic fluid, transfer of water and sodium to, 757.
- Anesthesia, evipal, Hering-Breuer reflexes under, 7.
- ANSBACHER, S. See MARTIN, ICHNIOWSKI, WISANSKY and ANSBACHER, 66.
- Anterior lobe of hypophysis and renal function, 584.
- Arterial reactions of finger to local cold, 680.
- ASHWORTH, C. T., E. E. MUIRHEAD and J. M. HILL. The effect of hypertonic plasma on the body fluids in normal experimental animals, 194.
- ASTWOOD, E. B. See TYSLOWITZ and ASTWOOD, 22.
- B**ALDWIN, E. DEF. See RICHARDS, COUNNAND, DARLING, GILLESPIE and BALDWIN, 115.
- BALE, W. F. See HAHN and BALE, 314.
- BARBORKA, C. J. See FOLTZ, IVY and BARBORKA, 79.
- BAVETTA, L. A. and H. J. DEUEL, JR. The effect of adrenalectomy on the absorption of hydrogenated cottonseed oil, corn oil, tributyrin and sodium butyrate, 712.
- BEAN, J. W. Specificity in the renin-hypertensinogen reaction, 731.
- BEATON, L. E. See MAGOUN and BEATON, 720.
- BEECHER, H. K. See MOYER and BEECHER, 7, 13.
- BEHRMANN, V. G. See FAY, BEHRMANN and BUCK, 716.
- BELCHER, D. See SHEDLOVSKY, BELCHER and LEVENSTEIN, 535.
- BENDER, M. B. and E. A. WEINSTEIN. Hypothalamic stimulation yielding adrenalin reversal effects, 376.
- Benzedrine, influence of, on work-decrement and patellar reflex, 392.
- BERK, J. E., J. E. THOMAS and M. E. REHFUSS. The effect of a cream meal on the acidity and neutralizing ability of the contents of the duodenal bulb in normal dogs, 285.
- , —, —, —. The reaction and neutralizing ability of the contents of the first part of the duodenum in normal dogs under fasting conditions, 369.
- , —, —, —. The reaction and neutralizing ability of the contents of the pyloric antrum and first part of the duodenum in normal dogs following an Ewald meal, 157.
- Bile salts and intestinal absorption of chloride, 340.
- BLAIR, H. A., A. M. WEDD and H. M. HARDWICKE. The normal pneumocardiogram, 523.



- Blood, cerebral,  $O_2$  and  $CO_2$  of, during diathermy, 177.
- , disappearance rate of tyrosine from, 460.
- flow, influence of posture on, 381.
- , renal, filtration rate and, after denervation, 38.
- plasma protein replacement after hemorrhage, 299.
- pressure in right auricle, 115.
- Blood pressure. *See* Hypertension.
- Blood pressure. *See* Hypotension.
- serum albumin and globulin levels, 306.
- , antigonadotropic, and gonadal development, 293.
- protein levels, adrenals and, 776.
- , swine, defective coagulation mechanism in, 355, 360.
- volume of finger tip, etc., variations in, 433.
- variations of finger tips, etc., in hypertension, 451.
- Body fluids, effect of hypertonic plasma on, 194.
- size, effect of, on energy exchange in work, 363.
- BOGART, R. *See* MÜHRER, HOGAN and BOGART, 355.
- BORKOVIC, E. J. *See* LOONEY and BORKOVIC, 177.
- BOZLER, E. The action potentials accompanying conducted responses in visceral smooth muscles, 553.
- The activity of the pacemaker previous to the discharge of a muscular impulse, 543.
- Brain, circulation, ciliary movement and, 223.
- , developing, respiration of, 600.
- , optic cortex and absolute threshold of vision, 463.
- BRASSFIELD, C. R. *See* GESELL, BRASSFIELD and HAMILTON, 604.
- BRAZDA, F. G. and J. C. RICE. Effect of fasting and feeding on the response of surviving tissues to quinidine, 386.
- BRIDGMAN, C. S. and K. U. SMITH. The absolute threshold of vision in cat and man with observations on its relation to the optic cortex, 463.
- BROWN, F. R. *See* NEWBURGER and BROWN, 746.
- BUCK, D. M. *See* FAY, BEHRMANN and BUCK, 716.
- BURCH, G. E., A. E. COHN and C. NEUMANN. A study by quantitative methods of the spontaneous variations in volume of the finger tip, toe tip, and postero-superior portion of the pinna of resting normal white adults, 433.
- *See* NEUMANN, COHN and BURCH, 448, 451.
- CAFFEINE, influence of, on recovery from fatigue, 79.
- Carbohydrate metabolism, influence of diethyl-stilbestrol on, 136.
- , pituitary and, 95.
- , protein and, influence of pituitary on, 128.
- Cardiac and vascular factors in hemorrhagic shock, 421.
- ganglion of *Limulus*, action of acetylcholine on, 182.
- idioventricular rhythms and fibrillation, 318.
- muscle changes after coronary occlusion, 474.
- Cardiac. *See* Heart.
- Carotid body anoxia, hypercapnia and asphyxia, 200.
- Cerebral blood,  $O_2$  and  $CO_2$  of, during diathermy, 177.
- differentiated irradiation, 783.
- Cerebrospinal circulation, ciliary movement and, 223.
- Chloride, intestinal absorption of, bile salts and, 340.
- CHU, H.-Y. Ciliary movement and circulation of cerebrospinal fluid within brain ventricles in larval and adult anurans, 223.
- Ciliary movement and cerebrospinal circulation, 223.
- Circulation, cerebrospinal, ciliary movement and, 223.
- Circulatory failure, adrenal steroids in, 567.
- responses to small amounts of adrenalin, 87.
- CLARK, S. L. *See* KENNEDY and CLARK, 140.
- Cloaca, rôle of, in water metabolism of chicken, 657.
- $CO_2$ ,  $O_2$  and, of cerebral blood during diathermy, 177.
- Coagulation mechanism, defective, in swine blood, 355, 360.

- CODE, C. F. See KOTTKE, CODE and WOOD, 229.
- COHN, A. E. See BURCH, COHN and NEUMANN, 433.
- See NEUMANN, COHN and BURCH, 448, 451.
- Cold, effect of, on forehead circulation, 692.
- , local, arterial reactions of finger to, 680.
- , vascular reactions of finger to, 669.
- COLLINS, D. A. See HAMILTON and COLLINS, 275.
- Coronary occlusion, histochemical changes after, 474.
- COSBY, R. S. See WERLE, COSBY and WIGGERS, 401.
- COURNAND, A. See RICHARDS, COURNAND, DARLING, GILLESPIE and BALDWIN, 115.
- CRAFTS, R. C. See LEVIN, LEATHEN and CRAFTS, 776.
- D**ANIEL, E. P. and E. M. HEWSTON. Vanadium—a consideration of its possible biological rôle, 772.
- DARLING, R. C. See RICHARDS, COURNAND, DARLING, GILLESPIE and BALDWIN, 115.
- DARROW, D. C. See DURLACHER and DARROW, 577.
- See DURLACHER, DARROW and WINTERNITZ, 346.
- DAVID, A. See GREENE, DAVID and JOHNSTON, 595.
- DEL POZO, E. C. See ROSÉNBLUETH and DEL POZO, 629, 699.
- Denervation, renal filtration and blood flow after, 38.
- Desoxycorticosterone, potassium-low diet and, renal size after, 346.
- DEUEL, H. J., JR. See BAVETTA and DEUEL, 712.
- Diathermy, O<sub>2</sub> and CO<sub>2</sub> of cerebral blood during, 177.
- Diet, potassium-low, and desoxycorticosterone, renal size after, 346.
- Dietary yeast, liver function and, in hyperthyroidism, 762.
- Diethylstilbestrol, influence of, on carbohydrate metabolism, 136.
- Diets, various, liver glycogen maintenance on, 746.
- DILL, D. B. See KNEHR, DILL and NEUFELD, 148.
- DINGLE, J. See GREGG, PRITCHARD, ECKSTEIN, SHIPLEY, ROTTA, DINGLE, STEEGE and WEARN, 250.
- DRILL, V. A. and H. W. HAYS. Studies on the relation of the liver function, pulse rate and temperature of hyperthyroid dogs to vitamin B<sub>1</sub> and yeast, 762.
- See SWINGLE, REMINGTON, DRILL and KLEINBERG, 567.
- DRINKER, C. K. See WARREN and DRINKER, 207.
- Ductus arteriosus, reactions of, 140.
- Duodenal acidity, fat ingestion and, 285.
- contents, pyloric and, reactions of, 157.
- Duodenum, descending, activity of, during nausea, 561.
- , reaction and neutralizing ability of, 369.
- DURLACHER, S. H. and D. C. DARROW. The effect of depletion of body potassium on the time of survival after nephrectomy and ureteral ligation, 577.
- , — and M. C. WINTERNITZ. The effect of low potassium diet and of desoxycorticosterone acetate upon renal size, 346.
- DUSCHATKO, A. M. See WIGGERS, DUSCHATKO and KORY, 87.
- E**BERT, R. V., E. A. STEAD, JR., J. V. WARREN and W. E. WATTS. Plasma protein replacement after hemorrhage in dogs with and without shock, 299.
- ECKSTEIN, R. W. See GREGG, PRITCHARD, ECKSTEIN, SHIPLEY, ROTTA, DINGLE, STEEGE and WEARN, 250.
- Edema, measurement of, in heart-lung preparation, 506.
- Electrocardiogram, nature of S complex of, 726.
- Electroencephalogram, effect of hypoglycemia on, 1.
- Electrolyte and water content of perfused heart, 515.
- Energy exchange in work, effect of body size on, 363.
- ESSEX, H. E. See HART and ESSEX, 657.
- Ether, effect of, on liver glycogen maintenance, 746.
- ETS, H. N. and I. M. FEINBERG. Some actions of indole on the dog, 647.
- Eye, parasympathectomized, effect of urethane on, 173.
- Eye. See Vision.

- FAT** absorption, adrenalectomy and, 712.  
 — ingestion and duodenal acidity, 285.
- Fatigue, double work periods in study of, 79.
- FAY, M., V. G. BEHRMANN and D. M. BUCK.  
 The parathyroids and the clearance of inorganic phosphate, 716.
- FEIGEN, G. A. See ALLES and FEIGEN, 392.
- FEINBERG, I. M. See ETS and FEINBERG, 647.
- Fibrillation, cardiac idioventricular rhythms and, 318.
- FINERTY, J. C. See KUPPERMAN, MEYER and FINERTY, 293.
- FLEXNER, L. B. and A. GELLHORN. The transfer of water and sodium to the amniotic fluid of the guinea pig, 757.  
 —. See GELLHORN and FLEXNER, 750.
- FOLTZ, E., A. C. IVY and C. J. BARBORKA. The use of double work periods in the study of fatigue and the influence of caffeine on recovery, 79.
- Food intake, activity and, after hypothalamic lesions, 609.
- Fructose, metabolism of, by eviscerated rat, 167.
- G**ANGLION, superior cervical, effects of veratrine on, 699.
- GARREY, W. E. An analysis of the action of acetylcholine on the cardiac ganglion of *Limulus polyphemus*, 182.
- Gastro-intestinal activity, traumatic shock and, 32.
- GELLHORN, A. and L. B. FLEXNER. Transfer of water across the placenta of the guinea pig, 750.  
 —. See FLEXNER and GELLHORN, 757.
- GELLHORN, E. and M. KESSLER. The effect of hypoglycemia on the electroencephalogram at varying degrees of oxygenation of the blood, 1.
- GERARD, R. W. See PEARCE and GERARD, 49.
- GESELL, R., C. R. BRASSFIELD and M. A. HAMILTON. An acid-neurohumoral mechanism of nerve cell activation, 604.  
 —, C. A. MOYER and J. B. MCKITTRICK. Some special instances of predominant chemical stimulation of the expiratory half-center, 486.
- GILLESPIE, W. H. See RICHARDS, COURNAND, DARLING, GILLESPIE and BALDWIN, 115.
- GILLIGAN, D. R. See LOWRY, GILLIGAN and HASTINGS, 474.
- Globulin levels, serum albumin and, 306.
- Glucose absorption and disposition in chick, 244.
- Glycogen maintenance, liver, on various diets, 746.
- GOLDEN, W. R. C. and C. N. H. LONG. Absorption and disposition of glucose in the chick, 244.
- Gonadal development after antigonadotropic serum, 293.
- Gonadotropic serum, anti-, and gonadal development, 293.
- GRANT, W. C. See SCHNEIDER and GRANT, 42.
- GREENE, J. A., A. DAVID and G. JOHNSTON. Production of insulin resistance in depancreatized dogs, 595.  
 — and G. W. JOHNSTON. The disappearance rate of tyrosine from the blood of dogs, 460.
- GREGG, D. E., W. H. PRITCHARD, R. W. ECKSTEIN, R. E. SHIPLEY, A. ROTTA, J. DINGLE, T. W. STEEGE and J. T. WEARN. Observations on the accuracy of the thermostromuhr, 250.  
 —. See SHIPLEY, GREGG and WEARN, 263.
- H**AHN, P. F. and W. F. BALE. Linear relationship between the circulating red cell mass and the venous hematocrit as determined with radioactive iron, 314.
- HAMILTON, A. S. and D. A. COLLINS. The homeostatic rôle of a renal humoral mechanism in hemorrhage and shock, 275.
- HAMILTON, M. A. See GESELL, BRASSFIELD and HAMILTON, 604.
- HARDWICKE, H. M. See BLAIR, WEDD and HARDWICKE, 523.
- HARRIS, A. S. and G. K. MOE. Idioventricular rhythms and fibrillation induced at the anode or the cathode by direct currents of long duration, 318.
- HART, W. M. and H. E. ESSEX. Water metabolism of the chicken (*Gallus domesticus*) with special reference to the rôle of the cloaca, 657.
- HASTINGS, A. B. See LOWRY, GILLIGAN and HASTINGS, 474.
- HAYS, H. W. See DRILL and HAYS, 762.

- Heart, frog, ion antagonism and, 351.  
 —, mammalian, action of ions on, 332.  
 — of *Limulus*, action of acetylcholine on, 182.  
 —, perfused, electrolyte and water content of, 515.  
 —, right, blood pressure in, 115.  
 Heart. *See* Cardiac.  
 Heart-lung preparation, measurement of edema in, 506.  
 Heat exchanges during recovery from undercooling, 105.  
 HEINBECKER, P., H. L. WHITE and D. ROLF. Relation of the adeno and the neurohypophysis to insulin sensitivity and sugar tolerance in the dog, 592.  
 —. *See* WHITE, HEINBECKER and ROLF, 584.  
 HEINEN, J. H. *See* HUGGINS, SCOTT and HEINEN, 467.  
 Hematocrit, jugular, radio-iron in evaluating, 314.  
 Hemorrhage and shock, renal humoral mechanism in, 275.  
 —, plasma protein replacement after, 299.  
 HENLE, G. and C. A. ZITTLE. Studies of the metabolism of bovine epididymal spermatozoa, 70.  
 Hering-Breuer reflexes under evipal anesthesia, 7.  
 HERTZMAN, A. B. and L. W. ROTH. The absence of vasoconstrictor reflexes in the forehead circulation. Effects of cold, 692.  
 — — —. The reactions of the digital artery and minute pad arteries to local cold, 680.  
 — — —. The vasomotor components in the vascular reactions in the finger to cold, 669.  
 HETHERINGTON, A. W. and S. W. RANSON. The spontaneous activity and food intake of rats with hypothalamic lesions, 609.  
 HEWSTON, E. M. *See* DANIEL and HEWSTON, 772.  
 HIATT, E. P. The effect of denervation on the filtration rate and blood flow in dog kidneys rendered hyperemic by administration of pyrogen, 38.  
 HILL, J. M. *See* ASHWORTH, MUIRHEAD and HILL, 194.  
 Histochemical changes after coronary occlusion, 474.  
 HOFF, H. E. *See* NAHUM, HOFF and KAUFMAN, 726.  
 HOGAN, A. G. *See* MUIRER, HOGAN and BOGART, 355.  
 HUGGINS, C., W. W. SCOTT and J. H. HEINEN. Chemical composition of human semen and of the secretions of the prostate and seminal vesicles, 467.  
 Hypercapnic stimulation, hypoxic and, at carotid body, 200.  
 Hypericium, etiology of, 650.  
 Hypertensinogen reaction, renin-, specificity in, 731.  
 Hypertension, oxidases, pressor amines and, 66.  
 —, volume variations of finger tips, etc., in, 451.  
 Hyperthyroidism, liver function and dietary yeast in, 762.  
 Hypertonic plasma, effect of, on body fluids, 194.  
 Hypoglycemia, effect of, on electroencephalogram, 1.  
 Hypophysis, anterior lobe of, and renal function, 584.  
 —, insulin sensitivity and sugar tolerance, 592.  
 Hypotension, hemorrhagic, and shock, 401.  
 Hypothalamic lesions, activity and food intake after, 609.  
 Hypothalamus, stimulation of, adrenalin and, 376.  
 Hypoxia, central stimulation of respiration during, 13.  
 Hypoxic and hypercapnic stimulation at carotid body, 200.  
 ICHNIOWSKI, C. T. *See* MARTIN, ICHNIOWSKI, WISANSKY and ANSBACHER, 66.  
 Idioventricular rhythms, cardiac, and fibrillation, 318.  
 Indole, actions of, 647.  
 INGELFINGER, F. J. and R. E. MOSS. The activity of the descending duodenum during nausea, 561.  
 Inositol, interrelationship of p-aminobenzoic acid and, 124.  
 Insulin resistance in depancreatized dog, 595.  
 — sensitivity and sugar tolerance, hypophysis, 592.  
 Intestinal absorption of chloride, bile salts and, 340.

- Ion antagonism and frog heart, 351.  
 Ions, action of, on mammalian heart, 332.  
 Irradiation, cerebral differentiated, 783.  
 IVY, A. C. See FOLTZ, IVY and BARBORKA, 79.
- JANES, R. G. and W. O. NELSON.** The influence of diethyl-stilbestrol on carbohydrate metabolism in normal and castrated rats, 136.
- JOHNSTON, G.** See GREENE, DAVID and JOHNSTON, 595.
- JOHNSTON, G. W.** See GREENE and JOHNSTON, 460.
- KAUFMAN, W.** See NAHUM, HOFF and KAUFMAN, 726.
- KEIL, F. C., JR. and W. S. ROOT.** The effect of the urethane of beta methylcholine chloride upon the parasympathetomized cat's eye, 173.
- KENNEDY, J. A. and S. L. CLARK.** Observations on the physiological reactions of the ductus arteriosus, 140.
- KESSLER, M.** See GELLHORN and KESSLER, 1.
- Kidney. See Renal.
- KLEINBERG, W.** See SWINGLE, REMINGTON, DRILL and KLEINBERG, 567.
- KNEHR, C. A., D. B. DILL and W. NEUFELD.** Training and its effects on man at rest and at work, 148.
- KORY, R. C.** See WIGGERS, DUSCHATKO and KORY, 87.
- KOTTKE, F. J., C. F. CODE and E. H. WOOD.** Urine dilutions and concentration tests in adrenalectomized dogs, 229.
- KUPPERMAN, H. S., R. K. MEYER and J. C. FINERTY.** Precocious gonadal development occurring in immature rats following a short-time treatment with antigonadotropic serum, 293.
- LAWSON, F. L.** The effect of sulfanilamide on the ability of rabbits and dogs to withstand high altitudes, 494.
- LEATHAM, J. H.** See LEVIN and LEATHAM, 306.
- See LEVIN, LEATHAM and CRAFTS, 776.
- LEVENSTEIN, I.** See SHEDLOVSKY, BELCHER and LEVENSTEIN, 535.
- LEVIN, L. and J. H. LEATHAM.** The relation of the pituitary, thyroid and adrenal glands to the maintenance of normal serum albumin and globulin levels, 306.
- , — and R. C. CRAFTS. The effects of adrenalectomy and replacement therapy on the serum protein levels of the cat, 776.
- Limulus heart, action of acetylcholine on, 182.
- Liver function and dietary yeast in hyperthyroidism, 762.
- glycogen maintenance on various diets, 746.
- LONG, C. N. H.** See GOLDEN and LONG, 244.
- LOONEY, J. M. and E. J. BORKOVIC.** The changes produced on the oxygen and carbon dioxide content of arterial and venous blood of the brain during diathermy therapy for general paresis, 177.
- LOWRY, O. H., D. R. GILLIGAN and A. B. HASTINGS.** Histochemical changes in the myocardium of dogs following experimental temporary coronary arterial occlusion, 474.
- Lungs, flow of lymph from, 207.
- Lymph, flow of, from lungs, 207.
- MAGOUN, H. W. and L. E. BEATON.** The salivatory motor nuclei in the monkey, 720.
- MARTIN, G. J.** The interrelationship of p-aminobenzoic acid and inositol, 124.
- , C. T. ICHNIOWSKI, W. A. WISANSKY and S. ANSBACHER. Oxidases, pressor amines and hypertension, 66.
- MAYERSON, H. S.** The influence of posture on blood flow in the dog, 381.
- McKITTRICK, J. B.** See GESELL, MOYER and McKITTRICK, 486.
- Metabolism, carbohydrate, influence of diethyl-stilbestrol on, 136.
- , —, pituitary and, 95.
- of bovine epididymal spermatozoa, 70.
- of fructose by eviscerated rat, 167.
- , protein and carbohydrate, pituitary and, 128.
- , water, of chicken, rôle of cloaca in, 657.
- MERTZ, E. T.** The anomaly of a normal Duke's and a very prolonged saline bleeding time in swine suffering from an inherited bleeding disease, 360.
- MEYER, R. K.** See KUPPERMAN, MEYER and FINERTY, 293.

- MOE, G. K. See HARRIS and MOE, 318.  
 —. See WOOD and MOE, 506, 515.
- MOSS, R. E. See INGELFINGER and MOSS, 561.
- Motor nuclei, salivatory, in monkey, 720.
- MOYER, C. A. and H. K. BEECHER. Central stimulation of respiration during hypoxia, 13.  
 — — —. Variability of the Hering-Breuer reflexes in the dog under sodium evipal anesthesia, 7.  
 —. See GESELL, MOYER and MCKITTRICK, 486.
- MUHRER, M. E., A. G. HOGAN and R. BOGART. A defect in the coagulation mechanism of swine blood, 355.
- MUIRHEAD, E. E. See ASHWORTH, MUIRHEAD and HILL, 194.
- Muscle potentials in a single volitional twitch, 743.  
 — tremors and temperature regulation in birds, 618.  
 —, visceral smooth, action potentials in, 553.
- Muscles, various, speed of responses of, 623.
- Muscular impulse discharge, pacemaker activity prior to, 543.
- NAHUM, L. H., H. E. HOFF and W. KAUFMAN. The nature of the S complex of the electrocardiogram, 726.
- Nausea, activity of descending duodenum during, 561.
- NECHELES, H. and W. H. OLSON. Experimental investigation on the effects of trauma and traumatic shock on gastrointestinal motility and secretions, 32.
- NELSON, W. O. See JANES and NELSON, 136.
- Nephrectomy, body K and survival time after, 577.
- Nerve cell activation, acid-neurohumoral mechanism of, 604.  
 — ganglion, effects of veratrine on, 699.
- Nerves, mammalian motor, speed of responses in, 629.
- NEUFELD, W. See KNEHR, DILL and NEUFELD, 148.
- NEUMANN, C., A. E. COHN and G. E. BURCH. A study by quantitative methods of the spontaneous variations in volume of the tips of the fingers and toes and postero-superior portion of the pinna of hypertensive patients and senile subjects, 451.  
 —, — — —. A study of the relationship between the pulse and alpha waves of the tips of the fingers and toes of five adults, 448.  
 —. See BURCH, COHN and NEUMANN, 433.
- Neurones, respiration of, 49.
- NEWBURGER, R. A. and F. R. BROWN. The effect of ether and starvation on liver glycogen maintenance after various diets, 746.
- O<sub>2</sub> and CO<sub>2</sub> of cerebral blood during diathermy, 177.
- ODUM, E. P. Muscle tremors and the development of temperature regulation in birds, 618.
- OLSON, W. H. See NECHELES and OLSON, 32.
- OSTER, R. H. See TOMAN and OSTER, 743.
- Oxidases, pressor amines and hypertension, 66.
- PACE, N. The etiology of hypericisism, a photosensitivity produced by St. Johnswort, 650.
- Pacemaker activity prior to impulse discharge, 543.
- p-aminobenzoic acid and inositol, interrelationship of, 124.
- Pancreatectomy, insulin resistance after, 595.
- PASCHKIS, K. E. Influence of anterior pituitary extract on protein and carbohydrate metabolism, 128.
- PEARCE, J. and R. W. GERARD. The respiration of neurones, 49.
- PETERS, H. C. The influence of bile salts on active intestinal absorption of chloride, 340.
- Phosphate clearance, parathyroids and, 716.
- Photosensitivity and St. Johnswort, 650.
- PINSON, E. A. and E. F. ADOLPH. Heat exchanges during recovery from experimental deficit of body heat, 105.
- Pituitary and adrenal cortex in resistance to cold, 22.  
 — and carbohydrate metabolism, 95.  
 — and protein and carbohydrate metabolism, 128.  
 —, relation of, to serum albumin and globulin levels, 306.

- Placenta, transfer of water across, 750.  
 Plasma, hypertonic, effect of, on body fluids, 194.  
 — protein replacement after hemorrhage, 299.  
 Pneumocardiogram, normal, 523.  
 Posture, influence of, on blood flow, 381.  
 Potassium, body, and survival time after nephrectomy, 577.  
 Potassium-low diet and desoxycorticosterone, renal size after, 346.  
 Pressor amines and hypertension, oxidases, 66.  
 PRITCHARD, W. H. See GREGG, PRITCHARD, ECKSTEIN, SHIPLEY, ROTTA, DINGLE, STEEGE and WEARN, 250.  
 Protein and carbohydrate metabolism, pituitary and, 128.  
 — replacement, plasma, after hemorrhage, 299.  
 Pulse and alpha waves of tips of fingers and toes, 448.  
 Pyloric and duodenal contents, reactions of, 157.
- QUINIDINE**, response of surviving tissues to, 386.
- RADIO-IRON** in evaluating jugular hematocrit, 314.  
 RANSON, S. W. See HETHERINGTON and RANSON, 609.  
 Reflex, patellar, work-decrement and, influence of benzedrine on, 392.  
 Reflexes, conditioned, 783.  
 —, Hering-Breuer, under evipal anesthesia, 7.  
 —, vasoconstrictor, absence of, in forehead, 692.  
 REHFUSS, M. E. See BERK, THOMAS and REHFUSS, 157, 285, 369.  
 REINECKE, R. M. The metabolism of fructose by the eviscerated rat, 167.  
 REMINGTON, J. W. See SWINGLE, REMINGTON, DRILL and KLEINBERG, 567.  
 Renal filtration and blood flow after denervation, 38.  
 — function after adrenalectomy, 229.  
 — —, anterior lobe of hypophysis and, 584.  
 — humoral mechanism in hemorrhage and shock, 275.  
 — size after potassium-low diet and desoxycorticosterone, 346.
- Renal. See Kidney.  
 Renin-hypertensinogen reaction, specificity in, 731.  
 Reproductive glands, male, chemistry of secretions of, 467.  
 Respiration during hypoxia, central stimulation of, 13.  
 — of developing brain, 600.  
 — of neurones, 49.  
 —, stimulation, chemical, of, 486.  
 — to record conditioned reflexes, 783.  
 RICE, J. C. See BRAZDA and RICE, 386.  
 RICHARDS, D. W., JR., A. Cournand, R. C. Darling, W. H. Gillespie and E. DeF. Baldwin. Pressure of blood in the right auricle, in animals and in man: under normal conditions and in right heart failure, 115.  
 ROBINSON, S. The effect of body size upon energy exchange in work, 363.  
 ROLF, D. See HEINBECKER, WHITE and ROLF, 592.  
 —. See WHITE, HEINBECKER and ROLF, 584.  
 ROOT, W. S. See KEIL and ROOT, 173.  
 ROSENBLUETH, A. and E. C. DEL POZO. Accommodation in mammalian motor nerves, 629.  
 — — —. The effects of veratrine upon the superior cervical ganglion, 699.  
 ROTH, L. W. See HERTZMAN and ROTH, 669, 680, 692.  
 ROTTA, A. See GREGG, PRITCHARD, ECKSTEIN, SHIPLEY, ROTTA, DINGLE, STEEGE and WEARN, 250.  
 RUSSELL, J. A. The anterior pituitary in the carbohydrate metabolism of the eviscerated rat, 95.
- SALIVATORY** motor nuclei in monkey, 720.  
 SCHNEIDER, E. C. and W. C. GRANT. Water intoxication of the frog (*Rana pipiens*), 42.  
 SCOTT, W. W. See HUGGINS, SCOTT and HEINEN, 467.  
 Secretions of male reproductive glands, chemistry of, 467.  
 Semen, chemical nature of, 467.  
 Serum protein levels, adrenals and, 776.  
 SHEDLOVSKY, L., D. BELCHER and I. LEVENSTEIN. Titrations of human seminal fluid with acids and alkalis and their

- effects on the survival of sperm motility, 535.
- SHIPLEY, R. E., D. E. GREGG and J. T. WEARN. Operative mechanism of some errors in the application of the thermostromuhr method to the measurement of blood flow, 263.
- See GREGG, PRITCHARD, ECKSTEIN, SHIPLEY, ROTTA, DINGLE, STEEGE and WEARN, 250.
- Shock, hemorrhage and, renal humoral mechanism in, 275.
- , hemorrhagic, cardiac and vascular factors in, 421.
- , —, hypotension and, 401.
- , traumatic, and gastro-intestinal activity, 32.
- SMITH, K. U. See BRIDGMAN and SMITH, 463.
- Sodium, transfer of water and, to amniotic fluid, 757.
- SPEALMAN, C. R. Ion antagonism and the frog heart, 351.
- The action of ions on the mammalian heart, 332.
- Sperm motility, survival of, 535.
- Spermatozoa, bovine epididymal, metabolism of, 70.
- Starvation, effect of, on liver glycogen maintenance, 746.
- STEAD, E. A., JR. See EBERT, STEAD, WARREN and WATTS, 299.
- STEEGE, T. W. See GREGG, PRITCHARD, ECKSTEIN, SHIPLEY, ROTTA, DINGLE, STEEGE and WEARN, 250.
- Steroids, adrenal, in circulatory failure, 567.
- Stimulation of expiratory half-center, 486.
- Sulfanilamide and high altitude, 494.
- SWINGLE, W. W., J. W. REMINGTON, V. A. DRILL and W. KLEINBERG. Differences among adrenal steroids with respect to their efficacy in protecting the adrenalectomized dog against circulatory failure, 567.
- TEMPERATURE, lower environmental, recovery from, 105.
- regulation in birds, muscle tremors and, 618.
- Temperatures, low, pituitary and adrenal cortex in resistance to, 22.
- Thermostromuhr, accuracy of, 250.
- , factors of error in application of, 263.
- THOMAS, J. E. See BERK, THOMAS and REHFUSS, 157, 285, 369.
- Thyroid, relation of, to serum albumin and globulin levels, 306.
- Thyroids, para-, and clearance of inorganic phosphate, 716.
- Tissues, surviving, response of, to quinine, 386.
- TOMAN, J. E. P. and R. H. OSTER. Muscle potentials accompanying a single volitional twitch, 743.
- Training and its effects on man at rest and at work, 148.
- Traumatic shock and gastro-intestinal activity, 32.
- TYLER, D. B. and A. VAN HARREVELD. The respiration of the developing brain, 600.
- Tyrosine, disappearance rate of, from blood, 460.
- TYSLOWITZ, R. and E. B. ASTWOOD. The influence of the pituitary and adrenal cortex on resistance to low environmental temperatures, 22.
- URETERAL ligation, body K and survival time after, 577.
- Urethane, effect of, on parasympathectomized eye, 173.
- VANADIUM, biological rôle of, 772.
- VAN HARREVELD, A. See TYLER and VAN HARREVELD, 600.
- Vasoconstrictor reflexes in forehead, absence of, 692.
- Vascular factors, cardiac and, in hemorrhagic shock, 421.
- reactions of finger to cold, 669.
- Veratrine and the superior cervical ganglion, 699.
- Vision and optic cortex, absolute threshold of, 463.
- WARREN, J. V. See EBERT, STEAD, WARREN and WATTS, 299.
- WARREN, M. F. and C. K. DRINKER. The flow of lymph from the lungs of the dog, 207.
- Water and sodium, transfer of, to amniotic fluid, 757.
- content, electrolyte and, of perfused heart, 515.
- intoxication of the frog, 42.
- transfer across placenta, 750.



- WATTS, W. E. See EBERT, STEAD, WARREN and WATTS, 299.
- WEARN, J. T. See GREGG, PRITCHARD, ECKSTEIN, SHIPLEY, ROTTA, DINGLE, STEEGE and WEARN, 250.
- See SHIPLEY, GREGG and WEARN, 263.
- WEDD, A. M. See BLAIR, WEDD and HARDWICKE, 523.
- WEINSTEIN, E. A. See BENDER and WEINSTEIN, 376.
- WERLE, J. M., R. S. COSBY and C. J. WIGGERS. Observations on hemorrhagic hypotension and hemorrhagic shock, 401.
- See WIGGERS and WERLE, 421.
- WHITE, H. L., P. HEINBECKER and D. ROLF. Effects of the removal of the anterior lobe of the hypophysis on some renal functions, 584.
- See HEINBECKER, WHITE and ROLF, 592.
- WIGGERS, C. J. and J. M. WERLE. Cardiac and peripheral resistance factors as determinants of circulatory failure in hemorrhagic shock, 421.
- See WERLE, COSBY and WIGGERS, 401.
- WIGGERS, H. C., A. M. DUSCHATKO and R. C. KORY. The circulatory response of the unanesthetized dog to small physiological quantities of adrenalin, 87.
- WILLS, J. H. Speed of responses of various muscles of cats, 623.
- WINDER, C. V. Combination of hypoxic and hypercapnic stimulation at the carotid body, 200.
- WINTERITZ, M. C. See DURLACHER, DARROW and WINTERITZ, 346.
- WISANSKY, W. A. See MARTIN, ICHNIOWSKI, WISANSKY and ANSBACHER, 66.
- WOOD, E. H. and G. K. MOE. Electrolyte and water content of the ventricular musculature of the heart-lung preparation with special reference to the effects of cardiac glycosides, 515.
- — — The measurement of edema in the heart-lung preparation, 506.
- See KOTTKE, CODE and WOOD, 229.
- Work, energy exchange in, effect of body size on, 363.
- Work-decrement and patellar reflex, influence of benzedrine on, 392.
- YEAST, dietary, liver function and, in hyperthyroidism, 762.
- ZITTLE, C. A. See HENLE and ZITTLE 70.



